Chapter 5

Bypass proteins from tree foliages

5.1 Introduction

There are several opportunities for using tree foliage high in protein to provide bypass protein to supplement a low protein forage diet given to ruminants. These include:—

- using the natural capacity of certain endogenous secondary plant compounds to bind with proteins and protect them from microbial attack. These complexes must then dissociate under acidic or alkaline conditions in the lower tract to provide digestible protein to the host.
- drying tree foliages under prescribed conditions. This is independent of whether tannins are present: other mechanisms may be active, e.g., a Browning reaction in the presence of reducing sugars in the leaf material

- harvesting plant foliages and treating them with chemicals and/or heat in order to produce a bypass protein through insolubilization of the leaf protein. Pelletting after treatment would be extremely advantageous.

5.2 Natural protection of leaf proteins

The literature has few references to the rôles of foliage protein as sources of fermentable N or escape or bypass protein.

Table 5.1: *Liveweight gains (LWG) of cattle grazing grass pasture either with or without access to* Leucaena *forage (Jones, 1994)*

	LWG (kg/head/d)		
Pasture	Nil	+ *Leucaena*	Access to *Leucaena* foliage
Native pasture	0.59	0.70	25% *Leucaena* on area basis
Native pasture	0.22	0.39	4 hours/day
Native pasture	0.18	0.33	25% *Leucaena* on area basis
Native pasture	-0.15	0.16	25% *Leucaena* on area basis
Native pasture	0.23	0.51	6% *Leucaena* on area basis
Native pasture	0.25	0.35	25% *Leucaena* on area basis
Native pasture	0.25	0.56	100% *Leucaena* on area basis
Cenchrus ciliaris	0.60	0.60	10 or 20 hours/week
Brachiaria	0.49	0.64	4 hours/day
Hyparrhenia rufa	0.27	0.35	10% *Leucaena* on area basis
Cynodon	0.29	0.41	4 hours/day
Dichanthium	0.21	0.50	20% *Leucaena* on area basis
Pennisetum	0.07	0.34	3 hours/day
Panicum	0.52	0.67	30% *Leucaena* on area basis
Panicum	0.18	0.37	30% *Leucaena* on area basis

Leucaena foliage has been reported to contain approximately 6.6% condensed tannin (Wheeler *et al.* (1995) and from Barry's work (Barry & Manley, 1984; Barry, 1983) with a forage legume, *Lotus*, these tannins could be expected to protect the proteins. This was confirmed in studies with sheep with re-entrant cannulae in the intestines where dietary protein from fresh *Leucaena* forage substantially escaped degradation in the rumen (Bamaulim *et al.* 1984). However, in these studies it was not determined whether this protein was available to the animal or whether it had been bound such that it avoided digestion in the small intestinal and the lower gut. Growth studies with cattle fed a variety of pastures with or without access to *Leucaena* (Jones, 1994) are surprisingly similar to results of supplements with MUMB to cattle on tropical pastures (see Table 5.1 on the preceding page and Chapter 3). These growth rates on pasture of cattle supplemented with *Leucaena* foliage are below those expected from a good quality bypass protein meal such as cottonseed meal fed to cattle on ammonia treated straw (see Figure 3.4 on page 43).

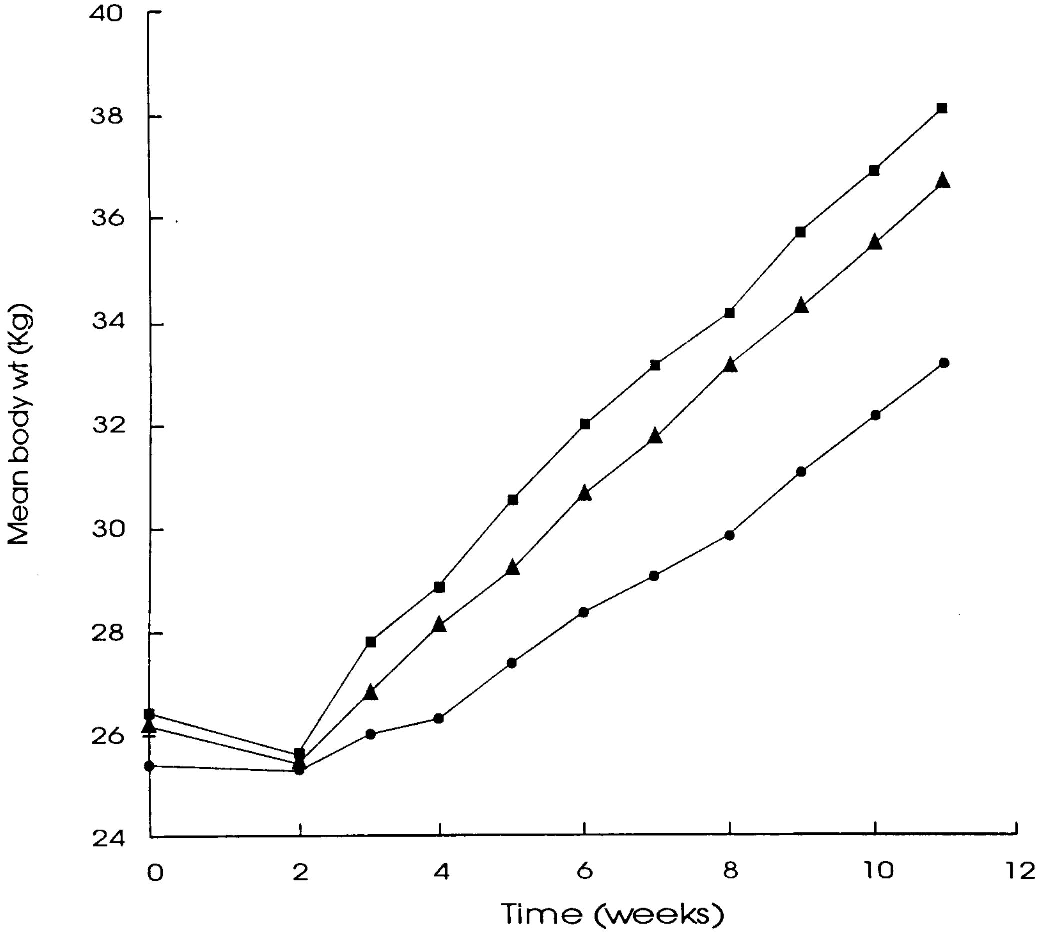
In all probability, *Leucaena* leaf protein (after drying) has some properties of both escape protein and fermentable N. Studies are required to understand these rôles in order to develop strategies that will promote substantial increases in cattle growth. A similar conclusion is also apparent for dried forage legumes such as lucerne (see Figure 5.1, page 75).

Jones (1994) also summarized the effects of supplementation with *Leucaena* foliage to dairy cows grazing pasture. Between 2 and 33% increase in milk yield apparently occurred in response to the cows' having access to *Leucaena*. Feeding MUMB to dairy cows at pasture in the tropics is summarized in Chapter 3 and similar milk yields (1 to 39%) were noticed.

The similarity in these results indicate that fresh *Leucaena* in tropical pastures possibly acts as an ammonia and mineral source to the rumen microbiota and that if the tannins do protect a proportion of the protein, this protein may not be available for digestion in the intestines. There seems to be little evidence that fresh tree leaf proteins provide escape or bypass protein with the exception on *Calliandra* spp. for supplementation of sheep, where a bypass protein response was apparent (see Table 4.5, page 66).

Similarly, *Erythrina poeppigiana* foliage was reported to have 42% crude protein and only 49% of this was soluble. However, it was dried before analysis.

Figure 5.1: *Effects of supplementation of lambs fed oaten chaff without supplements (●), or with 25% lucerne that had been sprayed with 1% xylose and dried before feeding (■), or untreated (▲) (Ball & Leng, 1994, unpublished).*



In some *Erythrina* species a greater percentage of the nitrogen is linked to acid detergent fibre suggesting extensive bypass protein in these species (Kass, 1994) but the question remains: is the protein that is protected in the rumen totally or partially available or unavailable to the animal? Where tree foliages after drying have been fed to goats on a basal diet of Napier grass, growth rate was stimulated over the use of the fresh foliage, but additional benefits have occurred when protected soyabean meal was also supplemented with the dry foliage (Table 5.2).

Table 5.2: *Effects of supplement of dry foliage and/or protected protein (formaldehyde treated soyabean meal [SBM]) source on growth rate of goats fed a basal Napier grass diet (Norton 1994*b, *after van Eys* et al. *1986).*

Napier grass intake (g/kg LWt/d)	Supplement (g/kg LWt/d)	LWt gain (g/d)
33.4	0	-1
29.4	4.1 *Gliricidia*	20
29.1	4.2 *Leucaena*	22
26.1	3.9 *Leucaena* + 2.7 g (SBM)	45
30.2	4.1 *Sesbania*	20
33.6	3.8 *Sesbania* + 2.7g (SBM)	52

5.3 Effects of drying fodder tree foliage

Supplementation with dried foliages from 5 tree species all produced greater liveweight gains in goats on a basal diet of poor quality forages than the same quantity of foliage fed fresh (see Table 5.3 on the next page). This is the type of response that might be related to a change in the solubility of the protein increasing the bypass protein content of the leaf meal. The reduction of anti-nutritive factors, however, might also have occurred. The former explanation seems more plausible, since the level of tannin would be quite different in the different species that were fed. A further possibility is that, in drying, the leaf meal undergoes a mild Browning reaction with the reducing sugars that would be invariably present in leaf materials and the protection has little to do with secondary plant components. However, that tannins may be implicated was shown by Ahn (1990); extractable tannin content of *Gliricidia* leaves was zero after

Table 5.3: *Comparison of the effect of tree foliage supplements fed fresh or after drying to goats (Norton, 1994, after Robertson, 1988).*

	Forage intake (g/kg LWt/d)				
	Tree foliage				
Supplement	Fresh	Dry	Basal forage	Dry matter digestion (%)	Growth rate (g/d)
Albizia chinensis	7.5		18.5	51	0
		7.5	16.5	44	54
Calliandra calothyrsus	7.5		18.5	47	24
		7.5	18.5	44	48
Gliricidia sepium	7.5		18.5	56	12
		7.5	15.5	47	42
Leucaena leucocephala	7.5		15.5	49	-18
		7.5	18.5	47	0
Sesbania sesban	7.5		14.5	53	0
		7.5	17.5	52	54

drying and when dried leaf meal was fed to sheep, straw intake increased and feed and N digestibility together with N balance were also increased. Recently, Dalzell (1996) has reported that drying reduces extractable condensed tannins to 25% that obtained from freeze dried samples.

If the concept that tannin-protein complexes were too strongly cross-linked in dried materials to provide a digestible bypass protein at the intestines is correct, then a mild Browning reaction in drying catalyzed by sugars in the leaf could make more protein available to the animal. A similar concept was recently promulgated by Broderick (1995) for forage legumes when dried. Drying might also remove anti-nutritive factors (Ahn, 1990; Norton, 1994), including the condensed tannins. That goats on low quality diets supplemented with fresh foliage respond to a source of bypass protein is clearly indicated in Table 5.2, page 76.

It may be costly to dehydrate or pellet leaf meals, and sun drying is a more accept-able and feasible alternative. However, application of heat might increasingly protect tree leaf meals as apparently occurs when lucerne meal is dehydrated at temperature increasing from 65°C to 160°C (Table 5.4, page 78).

5.4 Effects of adding chemicals

A number of chemicals when added to protein meals insolubilize the protein, thus protecting it from microbial degradation in the rumen. The best known of these is formaldehyde but the potential of formaldehyde to affect the health of humans or to produce carcinogens precludes its use in many situations, particularly in small rudimentary feed mills. In recent times the only alternative to formaldehyde is xylose which is found in acid waste from paper manufacture or is produced inexpensively from wood. Xylose is also readily produced by mild acid hydrolysis (e.g., steam pressure) from hemicellulose sources such as sugar cane bagasse or cottonseed hulls.

Table 5.4: *Effect of drying temperature on solubility and digestibility of N and N balance in lambs fed dried, chopped lucerne hay (Goering & Waldo, 1974).*

Drying temp (°C)	Solubility of N (%)	N digest (%)	N balance (%)
65	43	49	6.0
130	40	68	7.4
160	40	66	6.9
180	34	52	3.4

Molasses is a source of fructose and glucose that will react with the lysine of proteins to give a protected protein when the two are heated together. Molasses is potentially usable provided temperatures are not excessive, since glucose and fructose may easily produce a strong Browning reaction. However, xylose has much more potential as it forms a weak bond with lysine that is unlikely to produce over-protected proteins.

Lewis *et al.* (1988) demonstrated that mild heat with a small amount of xylose is very effective in protecting soyabean meal protein for cattle (Table 5.5) The effects of protecting lucerne forage protein with xylose on liveweight gain and wool growth of sheep is shown in Table 5.6, page 80.

Table 5.5: *Effects on liveweight gain of cattle of supplementing a basal forage/concentrate based diet with soyabean meal or soyabean meal treated with the xylose in sulphite liquor (SL) at 200° F for 2 hours (Lewis* et al. *1988).*

	LWt gain (g/d)
No supplement	591
+ 7% soyabean	673
+ 9% soyabean + 10% SL	823
+ 8% soyabean + 5% SL	841

In more recent work chopped lucerne hay treated with 1% xylose (prepared by hydrolysis of sugar cane bagasse) showed improved nutritional value when the lucerne was mixed at a rate of 25% of an oaten chaff diet (+ 1% urea and minerals) fed to lambs (Figure 5.1 on page 75).

5.5 Tree foliages as supplements or as basal diets

The developing countries in the tropics have, in general, little land for ruminant production *per se*, as crop production for human consumption is given the first priority. It is inconceivable that large tracts of land could be set aside for tree pastures where the trees exclude other forms of biomass. There are however, niches where this might be tolerated but it would be on either the farms of the rich (often absentee landlords), in the countries with low population densities, or where the fodder tree has multiple rôles which make it economic to produce. Australian farmers and farmers from a few other countries have some wide experience with using *Leucaena* as a component of pasture and also as the main harvestable biomass. Where such pasture have been established there seems to be real potential to grow cattle at around 200 kg/hd/year (about 700 g/head/day) with free access to *Leucaena* (Quirk *et al.* 1990). This is typical of, say, growth rates of cattle on good quality rye grass pastures under New Zealand farming conditions and/or irrigated pastures in Australia.

Table 5.6: *Liveweight gain and wool growth of sheep fed oaten chaff supplemented with either 220 g/d lucerne, lucerne treated with 0.5% xylose (Arreaza* et al. *1994).*

Supplement	Oaten chaff Intake (gDM/d)	LWG (g/d)	FCR (g/d)	Wool growth (g/d)
Nil	690	44	22	9
220 g lucerne	693	50	20	9
220 g treated lucerne	795	69	16	11

In Central America, trials with foliage from the fertilized mulberry as a feed for goats and a supplement for dairy cows have been very impressive, with growth and milk yields at high levels and cows achieving up to 18 litres of milk/day on forage supplemented with fresh mulberry foliage. However, under these circumstances mulberry must be cut and carried and fertilizer requirements are high (200 kg N/ha/yr) but this can be supplied as manure or green mulches (Benevides, 1994). To place this in perspective, the production rate on high intakes of tree foliages such as *Leucaena* and mulberry are only as good as those of cattle on straws treated with ammonia to increase digestibility and supplemented with 1–1.5 kg/d of cottonseed meal.

5.6 High density forage production from *Leucaena*

A recent major development with *Leucaena* shows great promise both for biomass production and for industrial processing of *Leucaena* leaf meal (Funes *et al.* 1996).

The development uses high density seeding rates (20 kg/ha) of *Leucaena* planted in a well prepared seed bed in rows at 0.50 metres apart. The seed is first heat treated and inoculated with a specific rhizobium.

The *Leucaena* is cut first at 10–15 cm above soil level when it has grown to a height of 1.2 metres. Thereafter 4–6 cuts can be taken per year when the plant reaches a height of 1 metre. The approximate yield under Cuban farming conditions without irrigation or fertilization is 12 tonnes DM/ha with 22–25% crude protein. In some experimental conditions 20 tonnes DM/ha have been achieved.

The forage may be fed fresh, or after sun-drying. There is great potential to fractionate the plants after drying into leaf meal (30% protein) and stems. The leaf meal then could be treated with molasses, hydrolyzed bagasse (xylose) or formaldehyde to protect its protein from microbial degradation in the rumen.

The combination of *Leucaena* in pasture and protected *Leucaena* leaf meal can be expected to have spectacular effects on animal productivity. The responses are relatively easily monitored using the established technology discussed later, i.e., measuring the milk yield and by purine excretion.

Figures 5.2 and 5.3 on the next page show high-density-forage: *Leucaena* growing in the tropical areas—Queensland and Cuba.

Figure 5.2: *Tree fodders (protein banks) for cattle production in the tropics.*

A. Leucaena leucocephala, *growing at Biloela, Queensland. Tracks have been left during planting to facilitate grazing.*

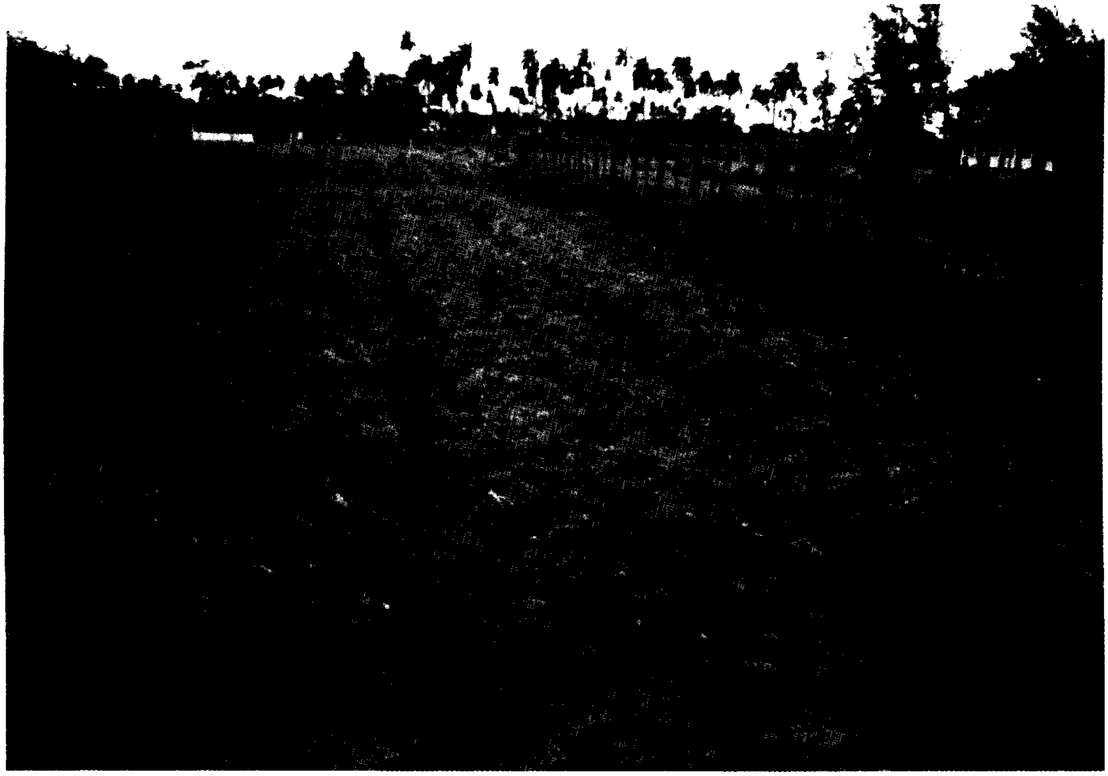
5.7 Assaying the nutritional value of protein in leaf foliages or meals

The operative questions that have arisen consistently throughout these discussions have been:—

1. how can the level of bypass protein in leaf meal be assessed?
2. when are the rumen micro-organisms efficient in growth and digestion of forage?

Figure 5.3: *Tree fodders (protein banks) for cattle production in the tropics.*

B. Leucaena, *high density plantings in Cuba. Used as cut and carry forage for goats. The* Leucaena *is allowed to grow to 1 metre before harvesting. About 6 cuts are taken annually.*



Both these questions are now able to be resolved, at least in the laboratory. There have been many attempts to predict the mechanisms of utilization of leaf proteins, including chemical analysis of acid detergent-N, protein solubility in buffers and ammonia production when the leaf meal is incubated with rumen fluid. The best that can be concluded from these studies is that they give an indication of the form of the protein. However, a major problem is apparent for any analytical approach: drying causes unknown changes in many leaf meals from different sources.

In the last few years it has been apparent that wool production (in sheep with a good capacity to grow wool) and milk yield (in high yielding dairy cows or goats) can be used as a bio-assay of amino acid absorption in ruminants. Essentially, these animals, on a diet of high digestibility forage and with a rumen provided with all microbial nutrients, will respond in wool/milk production to inputs of extra protein. Response curves to a standard protected protein can be used as the control and the response to an input of leaf meal will be indicative of the level of bypass protein.

Purine excretion in urine has long been viewed as a potential microbial marker. It is now known that the amount of microbial biomass digested in the intestines is highly correlated with total purine excretion. Thus, purine excretion in urine, combined with the protein assay using wool growth or milk yield will give an estimate of the extent of bypass of the protein and any improved production of microbial cell biomass.

The assay system would appear to be simple when working with animals in pens but quite difficult to apply to grazing animals. The intake of forage is critical under the latter conditions and there are no methods for satisfactorily measuring forage intake from mixed pastures or silvipastoral systems.

The wool growth assay appears to be quite a logical one where wool growing sheep are available. The basis of the assay is that wool growth is highly dependent on the protein digested in the intestines and therefore the amino acid supply to the animal (Leng *et al.* 1983). However, its weakness is that, more correctly, wool growth is highly dependent on the S-amino acids in the mixture of amino acids absorbed; but this should not be a major constraint as S-amino acid contents of leaf meals do not vary greatly. An assay with cows using milk yield would be quite costly to establish, but the use of goats, particularly those that breed all year round should be quite possible.

An approach to such an assay might be as follows:—

Experimental animals

These should be sheep or goats adjusted to a basal low protein diet (chopped forage with 55–60% digestibility) supplemented with minerals/urea. Minerals/urea may be best given as a multinutrient block to which the animals have been previously trained to consume small amounts at regular intervals.

The animals are then divided into groups (minimum 4 but optimum 6–10) on an equal mean live weight basis, animals that consume less than 90% of the average intake of forage are excluded.

Treatments

The following feeding trials are then undertaken:—

Treatment	**Diet**
2.	Diet-C + 100 g leaf meal
3.	Diet-C + 150 g leaf meal
4.	Diet-C + 200 g leaf meal
5. Control	Diet-C + 200 g of the same chopped forage

The animal intake is restricted to a level that is 200 g below the average intake of all animals.

Parameters

Wool growth can be measured using a small area on both sides of the animal that is clipped after three weeks on the diet and then again three weeks thereafter. Similarly a dye band can be placed on the wool at the surface of the skin at these times. At the end of the six week feeding period and over one week, total urine collection will be made daily and purine analysis determined on the accumulated sample to provide a mean daily excretion rate.

Any animal refusing feed for more than one day should be discarded from the statistical analysis.

Comments

The same experiment could be carried out with lactating goats or cows, but using initial two week period on the basal (control diet) as a covariance to adjust milk yield over the three weeks following transfer to the treatment groups.

Simultaneous studies with freshly harvested or dried foliages would quickly resolve some of the questions associated with responses to dried *versus* fresh foliage. a major problem here is that there can be substantial differences between the goat and sheep because of the former's major ability to detoxify secondary plant compounds.

5.8 Conclusions

Although low levels of condensed tannins in tree leaf protein consumed by ruminants appear to increase dietary protein entering the intestines, there must be considerable doubt about its quantitative significance in terms of extra protein available to the animal. Most studies with *Leucaena* as a supplement to ruminants on poor quality forages appear to support growth rates in cattle that are similar to those recently reported for the use of MUMB with cattle on tropical grasslands. This suggests a major rôle of the leaf meal supplement in stimulating rumen fermentative digestion and the efficiency of forage utilization. If this is tested and found to be true then the effectiveness of *Leucaena* or other tree fodders to improve cattle production from forages resides in its ability to provide the critically deficient nutrients required by the rumen microbiota. It is probably necessary to harvest and process tree foliage to meet the second rôle, i.e., the need for extra dietary amino acids by the animal once the rumen conditions have been optimized. However, where tree plantations under the prevailing climate, produce more biomass than pasture this will be a key feature for the acceptance of forage trees as a sole biomass feed. This will hardly be economic in most developing countries in the tropics even where the tree has multiple rôles.

Heat or drying appear to produce bypass proteins from the soluble proteins of tree foliages but the mechanisms by which this occurs is still not clear and may not necessarily involve reactions with tannins.

There appears to be considerable potential to develop bypass protein concentrates from leaf meals using simple low temperature drying in the presence of reducing sugars.

5.9 Postscript

The use of trees as components of diets is a widespread practice in many tropical countries. In Sri Lanka, for instance, some 200 tree foliages are fed to both large and small ruminants in an *ad hoc* way when available.

Tree foliages have assumed only slight importance in animal nutrition in developing countries, except where only browse is available. The largely undirected use of tree foliage is perhaps the main reason for the obvious lack of development of trees as feed for ruminants. In some cropping areas, other, more easily obtainable resources preclude their use, whereas the difficulties of managing plantations, harvesting and storage make them difficult to accept by pastoralists. Without education of the small farmer, tree foliages are not likely to be used extensively unless the foliages are produced in the appropriate form and provided as packages to farmers for their animals.

A case in question is the use of *Prosopis* pods in Brazil. Use of these in animal production has been only stimulated by the development of strategies to organize collection, marketing and processing of the pods. Trees will assume a much greater significance in the future because of the associated environmental advantages of planting trees. There should be a greater use of tree foliages as supplements to ruminants, particularly as the demand for animal products increases in the developing countries. Research and extension is needed to ensure their rational use, particularly as supplements to crop residues, which will be the major resource in the future for ruminants in developing countries.