EVALUATION OF DRIED LEUCAENA LEAF FROM MALAWI

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ABSTRACT

Abstract

The <u>in vivo</u> digestibility of two grades of sun/air dried Leucaena leucocephala leaf cv. Peru of Malawi origin was investigated using sheep in metabolism crates and fed at maintenance level. Both grades of leaf were pelleted before feeding, the B grade pellets being manufactured from unground material. The crude protein, crude fibre and mimosine levels were reported (g/kg DM):- Grade A pellets 289; 106; 11 Grade B pellets 188; 228; 16.

The five treatments, each with three replicates used in the experiment, together with feeding levels (g/head/day) were as follows:-

Treatment 1. Hay (500 g)

Treatment 2. Hay (350 g) with Leucaena A pellets (296 g)

Treatment 3. Leucaena B pellets (726 g)

Treatment 4. Hay (350 g) with Leucaena B pellets (363 g)

Treatment 5. Leucaena A pellets (592 g)

The 10 day digestibility trial followed a three week period of adaption during which levels of Leucaena intake were slowly increased. Despite this adaptive period, considerable health problems were encountered. Toxicity reactions were extremely variable, but most acute in Treatment 5, in which two sheep died. Some fleece shedding and digestive disturbances were experienced with the other leucaena based diets.

Metabolisable energy, organic matter and crude protein digestibility coefficient with standard deviations for grade A pellets of 8.7 ± 1.25; $0.54 \pm .043$; $0.61 \pm .037$ were calculated by difference, and for grade B pellets of 5.8 $\pm$ .07; 0.43 $\pm$ .014; 0.47 $\pm$ .064 were determined directly.

An apparent depression in hay digestibility in the presence of leucaena is reported and, in consideration of the health problems, the validity of all the values is discussed.

Toxicity reactions were investigated and post mortem findings and blood cell count data are presented.

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INTRODUCTION

EVALUATION OF DRIED LEUCAENA LEAF FROM MALAWI

Introduction

Agriculture in the Southern African Republic of Malawi has traditionally been concerned with the growing of arable crops such as maize, groundnuts, cotton and tobacco. However, in recent years there has been a greater awareness amongst smallholders of the potential profitability of live-stock enterprises. A number of successful stall-feeding schemes for fattening beef cattle have been initiated (THOMAS and ADDY, 1977A) and ADDY and THOMAS (1977A) have demonstrated the potential that exists for beef production from grazed leys with supplementation. To support satisfactory levels of animal production tropical pastures need to be both productive and of a relatively high nutritive value. The production of dry matter depends upon adequate supplies of soil nitrogen, but throughout much of the country the outstanding soil nutrient deficiency is nitrogen. Although good responses have been obtained from fertiliser nitrogen (ANON, 1970), it is unlikely that intensive nitrogen fertilisation will be an economic method of improving pasture production. The main nitrogenous fertilisers used in Malawi increased in price by over 180 per cent between 1971 and 1975, and this trend is likely to continue in the foreseeable future (THOMAS, 1975). Moreover, in winter (dry season) from May to October a substantial decline occurs in the nutritive value of grasses, and mature herbage has a high crude fibre content, low digestibility and low crude protein level. The latter is well below the critical value of 7 per cent reported by MILFORD and MINSON (1966). In the absence of supplementation lineweight losses of between 32 and 40 kg per beast have been observed (ANON; 1961 and 1963) during this period in cattle grazing unfertilised pastures.

In an attempt to overcome these constraints attention has been focused on the establishment of pasture legumes in cultivated pastures and rangeland. From evidence accummulated over a number of decades in Australia and other African countries legumes can (i) make a valuable contribution to the nitrogen economy of pastures (THOMAS, 1973) (ii) improve the nutritive value of pastures (MILFORD and MINSON, 1966) and (iii) increase animal production (NUTHALL and WHITEMAN, 1972). One such promising legume is <u>Leucaena Leucocephala</u> (Lam.) de Wit (≡ <u>Leucaena glauca</u> (L) Benth.), commonly known as leucaena. The importance of this plant in tropical pasture has been reviewed by Hill (1971). In Malawi its popularity is increasing amongst smallholders as a potentially inexpensive source of protein for cattle nutrition. Leucaena is easily established from seed or stumps (Savory and Thomas 1977). In many tropical countries, the plant is grazed <u>in situ</u> but in Malawi leucaena is cut, the branches sun dried and the leaf harvested for the production of meal, which is marketed in two main grades, A and B (Savory 1974 Personal communication).

Although much is known of the agronomy of the species little data are available on the digestible nutrient status of leucaena. <u>In vitro</u> determinations conducted at Edinburgh University on Grade A leaf, which has been compared with Medicago sativa (Lucerne) (Anon 1975) were lower than anticipated, and no <u>in viro</u> values were available. Accordingly, a preliminary investigation was carried out to evaluate the apparent digestibility of dried leucaena meal produced in Malawi.

REVIEW OF LITERATURE

a. Leucaena leucocephala

b. Evaluating food digestibility

Review of Literature

1. General Feature

A. Leucaena Leucocephala

Leucaena is a leguminous tree or arborescent shrub which may grow to a height of 10 m. It is characterized by bi-pinnate leaves, lanceolate leaflets, white flowers in globular heads, strap-shaped pods and brown shiny seeds. The plant is native to Mexico (Dijkman 1950) but has now spread to many parts of the tropics. Prior to the 1950's it was mainly cultivated to provide shade and maintain soil fertility in plantations. Only in comparatively recent times has leucaena been seriously considered as a livestock feed.

Leucaena has a strongly developed root system enabling the plant to withstand long periods of droughts. It is tolerant of a wide range of soil and climatic conditions (Hill 1971) and is capable of fixing substantial quantities of symbiotic nitrogen (Hutton and Bonner 1960). Leucaena is very acceptable to livestock and produces high yields of dry matter (DM) and crude protein (CP). Hill (1971) quotes annual dry matter yields up to 20516 kg/ha and crude protein yields of 1900 to 3600 kg/ha depending on climate, method of utilization, cultiver and fertilizer application.

2. Nutritive value

A. Digestibility

Few invesitgations have been undertaken to determine digestibility values for leucaena. Gohl (1975) quotes data for dry matter, crude protein and crude fibre of 60 per cent, 65 per cent and 35 per cent respectively. In Australia, young leucaena shoots were found to be 68 to 70 per cent digestible when fed to goats previously dosed with rumen fluid from steers grazing the plant (Jones, pers. comm. 1977). In vitro determinations have been conducted in Edinburgh with Grade A leucaena leaf from Malawiand a Digestible organic matter in the dry matter (D value) of 53.0 recorded by Thomas and Addy (1977b).

B. Chemical composition.

Table 1 summarises analyses of leucaena and lucerne. The high contents of crude protein and nitrogen free extract.

Table 1 Chemical composition of dried leucaena leaf (Malawi) and lucerne (Thomas, unpublished data 1976)

	Analysis of Dry Matter (%)						
	CP	MAD Fibre[1]	Ash	Ca.	P.	NFE[2]	β-Carotene
Leucaena leaf	28.8	20.4	11.0	2.5	0.23	41.2	536
Lucerne	26.9	21.7	16.6	3.2	0.36	38.8	253

1. Modified acid detergent fibre
2. Nitrogen free extract

(NFE) underline the considerable potential of leucaena as a forage. D'Mello and Thomas (1978) have also determined the amino-acid content of leucaena leaf. The amino-acid pattern compared favourably with data quoted by Hill (1971) except that the total sulphur amino acid content of the Malawi material was considerably higher.

C. Toxicity

An amino acid of considerable importance in leucaena is mimosine, β-(1-(3(hydroxy-4-pyridone))-α-amino-propionic acid, which was first isolated by KOSTERMANS (1946) from leucaena seed during investigations into loss of hair in young women who had eaten the seed. OWEN (1958) has reviewed the extensive literature on the toxic effects of leucaena and reported hair loss in horses from mane and tail, and loss of hair in pigs. In non-ruminants mimosine has now been conclusively shown (Yoshida, 1944; Hylin and Lichton, 1965) to cause depilation and infertility. Work with mice (Montagna and Yun; 1963) and plants (Pritchard and Court, 1968) indicated that mimosine acts as a mitotic inhibitor.

Reports in the literature on the toxicity of leucaena to ruminants is conflicting. OWEN (1958) reported that in Indonesia, Hawaii and the Bahamas no toxic effects occurred in goats, Holstein cows or sheep feed fresh leucaena leaves for varying periods of time. In Malawi Angoni steers fed dried leucaena leaf in quantities up to 4.5 kg per head per day over an eight week period exhibited no symptoms of toxicity (THOMAS and ADDY, 1977b). However, drying is known to reduce the mimosine content of leucaena (HILL, 1971).

On the other hand toxic symptoms in both sheep and cattle have been reported from Australia and Papua New Guinea. Hegarty

Schinckel and Court (1964) fed Merino wethers and cross-bred ewes 6 to 800 g per head per day of sun-dried leucaena leaf containing 2 per cent mimosine. Voluntary intake and body weight was reduced whilst severe fleece loss occurred some 6 days after feeding started. In a second experiment three sheep were fed diets containing either 100, 70 or 50 per cent leucaena. In all cases the leucaena was ground and pelleted. Sheep began shedding their fleece within 6 days of the beginning of the feeding period. The animal on pure leucaena died on the tenth day. It was suggested that leucaena toxicity was enhanced by grinding followed by pelleting. This would have resulted in an increased rate of passage of the ingesta through the alimentary tract and a reduction in exposure time to detoxification in the rumen. Hegarty and his co-workers were able to prevent fleece loss on pure leucaena diets by introducing a preliminary twenty week adaption period in which a mixture of lucerne chaff and crushed oats was gradually substituted by leucaena. It was suggested that conditioning of sheep to leucaena resulted from adaption of the micro-organisms in the rumen and their capacity to degrade mimosine rather than to any adaptive tolerance on the part of the animal. Similar results were achieved by the addition of ferrous carbonate to leucaena rations.

Jones, Blunt and Holmes (1976) reported enlarged thyroid glands in cattle grazing leucaena pastures at three sites in Australia and Papua New Guinea. Associated effects of prolonged leucaena grazing included low liveweight gain, excessive salivation and hair loss. These effects differed markedly from the acute effects of mimosine toxicity resulting from feeding pure diets of leucaena to sheep (Hagarty __et al__, 1964). Hair loss, excessive salivation and weight loss only occurred after periods of several months on leucaena pastures; a period which should have conditioned the animals, via adaption of the rumen micro-organisms, to degrade mimosine and so avoid its toxic effects. The poor liveweight gains of these animals on leucaena for eight to twelve months compared with satisfactory gains of animals previously unaccustomed to leucaena suggested a cumulative effect of the plant on animal performance. Holmes (1976) showed that these effects were produced even when animals received supplementary iodine. Hegarty, Court, Christie and Lee (1976) further showed that in rats and mice goitrogenic effects could not be reproduced with mimosine, but 3, 4-dihydroxypyridine (DHP), to which a major part of the ingested mimosine is converted in the rumen, was a potent goitrogen. They suggested that goitres in cattle were associated with the absorption of DHP formed in large quantities in the rumen from ingested mimosine.

D. Animal Production

Few grazing trials measuring yield of animal products from leucaena have been conducted. In Hawaii, Henke, Work and Burt (1940) grazed Aberdeen Angus steers on leucaena pasture over three trial periods of 246, 368 and 444 days. Liveweight gains ranged from 0.20 to 0.52 kg per head per day. Weight gains were closely related to rainfall during the experimental periods. Later, Henke and Morita (1954) used leucaena in a trial with Holstein dairy cows over a five-year period. They obtained more milk, with a higher fat content, than from cows of the same breed fed Pennisetun purpureum (Napier grass) and concentrates. Also in Hawaii, Furr (1965) compared production of grade Hereford with Santa Gertrudis X Hereford and Charolais X Hereford steers on a Tripsacum laxum (Guatemala grass) - leucaena pasture for 158 days. The average daily weight gain was 0.54 kg for the Hereford steers and 0.63 kg for the cross-breds.

The value of dried leucaena leaf as an alternative protein supplement to expensive oil-seed cakes has been investigated in Malawi in both the grazing and stall-feeding situations. Thomas and Addy (1977b) fed dried leucaena leaf during winter (dry season) to Angoni yearling steers grazing fertilised Chloris gayana (Rhodes grass) foggage in its second year. In another treatment cottonseed cake was fed at a rate of 0.34 kg per head per day. The level of leucaena given was such as to supply the same amount of crude protein as the cottonseed cake. During the experimental period, animals on Rhodes grass foggage only gained 4.5 kg compared with 19.1 and 20.9 kg respectively for the Rhodes grass plus Leucaena and Rhodes grass plus cottonseed cake treatment. No significant differences were recorded between supplements.

The interchangeability of cottonseed cake and leucaena as a protein source for cattle was further demonstrated in a wet season stall fattening trial, in which similar weight gains were achieved from two high protein supplements compounded from maize and maize bran with either cottonseed cake or leucaena (Thomas and Addy 1977(a)). In a further trial the efficiency of leucaena as a protein source for the winter period was demonstrated when a maize bran supplement was compared with supplements containing maize bran plus leucaena at 20 per cent or 50 per cent. The supplements were fed *ad libitum* to Angoni steers receiving restricted maize stover. Liveweight gains were increased from 0.8 kg/day to over 1 kg/day by the inclusion of leucaena in the supplement and conversion efficiency was also improved.

Evaluating Feed Digestibility

The apparent digestibility of a feed (generally referred to as digestibility) is that proportion not excreted in the faeces and which is therefore assumed to have been absorbed by the animal (McDonald et al 1973). It is of major importance in assessing the value of that feed to the animal.

Digestibility can be determined in vivo or in the laboratory by the two stage in vitro technique developed at the Grassland Research Institute Hurley. (Tilley, Deriaz and Terry 1960; Tilley and Terry 1963). This bio-assay, which has consistantly shown the highest correlation with in vivo values, involves the incubation of the feed with rumen liquor followed by digestion with pepsin, and is satisfactory for both temperate and tropical pastures (McLeod and Minson 1969a) and grass-legume mixtures (McLeod and Minson 1969b) provided that the results are standardized with similar samples of known in vivo digestibility, as the relationship between the two assessments of digestibility varies with different feeds. Generally at low digestibilities, in vitro values are very much lower than in vivo results (Minson-personal communication). In Australia, the Commonwealth Scientific and Industrial Research Organization have produced over 900 standard samples of known in vivo sheep digestibility, with which to standardize in vitro results (Minson personal communication).

The Digestibility Trial

The use of the digestibility trial for evaluating feeds dates back more than one hundred years (Schneider and Flatt 1974) but methods of calculating digestibility coefficients of individual nutrients have remained remarkably uniform. Where the food being investigated can be fed as the sole diet, calculation of its digestibility is straight forward. However, where mixed diets have to be fed, for example concentrates and roughage, calculation of the digestibility of one of the components of the diet becomes complex. Under these circumstances, the most common method employed is calculation of digestibility by difference (Schneider and Flatt 1974).

In the difference method, a basal feed is fed alone and its digestibility determined. It is then fed with the supplement of unknown digestibility, and the digestibility of the mixture established. It is assumed that there are no associative effects between the feeds in the mixed diet and that the digestibility of the basal diet has remained unchanged. The digestibility of the supplement is then calculated by difference.

The problems encountered in this calculation, and the validity of the assumptions made with regard to the digestibility of the basal diet when fed with a supplement, have been discussed by Frederiksen (1975) who concluded that the accumulation of one sided errors in the calculated digestibilities by difference, together with the unpredictible nature of associative effects made the method unsuitable for use in digestibility trials. Schneider and Flatt (1974) also emphasise that the method is, at best, approximate, and is utilized only where the nature of the feed precludes it from being fed as the sole diet.

Factors affecting digestibility of a feed

Food composition. The digestibility of a feed is closely related to its chemical composition, and, more particularly, to its crude fibre content. Although the crude fibre fraction does not include all the cell wall constituents, it does provide a guide to the concentration of these constituents and their degree of lignification (McDonald *et al* 1973).

The apparent digestibility of crude protein, and its dependancy upon the crude protein content of the food is also discussed by McDonald *et al* 1973). When low protein food is ingested, the constant loss of metabolic faecal nitrogen, estimated at approximately 3 g per 100 g of dry matter eaten, will impose a limit on the possible apparent digestibility of the crude protein content of that food.

Pelleting. Pelleting is generally preceeded by grinding and may cause a considerable depression in feed digestibility. Ground roughages pass through the rumen faster than long or chopped material, and are therefore exposed to the rumen micro-organisms for a shorter period of time, resulting in a less complete fermantation of the fibrous components (McDonald *et al* 1973). The subject was reviewed by Minson (1963) who reported a mean depression of 3.3 digestibility percentage units from twenty six digestibility trials.

Level of feed. The bulk of evidence in the literature indicates that a depression in digestibility of a feed accompanies an increase in the level of feed intake. Mitchel (1942) stated the general belief that published digestion coefficients were too high for use at productive levels of feeding by at least five percentage units because, in general, they had been obtained at maintenance or only slightly super maintenance levels of feeding.

However, where mixed feeds of varying palatability are fed at predetermined ratios, maintenance feeding is one of the most satisfactory ways of ensuring the complete intake of all dietary components.

The Use of Sheep in evaluating cattle feeds. For reasons of convenience and economy, sheep have been commonly used to measure the digestibility of feeds primarily used in cattle rations. In the United States, for instance, more than seventy per cent of the ruminant digestibility trials have been conducted with sheep (Schneider and Flatt 1974). The question of inter-species adaptability of digestibility data has been studied by a number of workers. Cipolloni (1951) analysed 1912 digestibility trials conducted on 27 feeds by 386 authors and was unable to conclude that sheep have poorer or better digestibility powers than cattle, or that the two species were identical in this respect. Playne (1970) has shown that sheep rank feeds in a similar order of digestibility and voluntary intake as do cattle although the absolute values may differ. In the view of the Advisory Committee to the Rowett Research Institute (1971) which cited some eighty reports on the subject, measurements of feed digestibility using sheep were, in most cases, applicable to cattle; the major exception being grain based feeds.

Accuracy of trials

Analysis of all the digestibility trials using sheep carried out at the Edinburgh School of Agriculture over the last twenty years (Franklin 1977 - personal communication) has shown an overall variance in digestibility of hay type feeds of 4.3 with 26 degrees of freedom. This is considerably higher than the variation in digestibility of grass feeds (1.885 with 60 degrees of freedom) and indicates an increase in the variability between individuals in their ability to digest feeds of lower quality.

MATERIALS AND METHODS

Materials and Methods

1. Location

The trial was carried out in the Sheep Metabolism Unit of the Department of Agricultural Biochemistry, Edinburgh University School of Agriculture, during July and August 1977.

2. Experimental animals.

15 four to five month old Suffolk X Scottish half bred castrated sheep weighing between 28 and 32 kg. liveweight were used. They had been dosed with an anthelminthic before the trial.

3. Experimental design

A completely randomised design with 3 fold replication and five experimental treatments (T) as shown in Table 2. The feeds under investigation were fed at 50 per cent and 100 per cent of the diets, in case of toxicity reactions in the experimental animals.

Table 2 Experimental Design

Treatment No.	Feedstuff	Estimated provision of Mm* requirement (%)	Sheep numbers
T1	Hay	100	233, 157, 334
T2	Hay Leucaena A	50 50	346, 406, 487
T3	Leucaena B	100	110, 220, 499
T4	Hay Leucaena B	50 50	135, 244, 206
T5	Leucaena A	100	483, 105, 108

*Mm The metabolisable energy requirement for maintenance.

4. Feedstuffs

LEUCAENA. Two grades (A and B) of sun/air dried leucaena leaf, variety PERU, were flown from Malawi to Scotland and pelleted on a Christy and Norris Pressfeed No. 10 machine.

A grade Leucaena : Pure greenleaf, dry enough to crush easily by hand. All twigs, pods, flower heads and rachises removed, together with most of the rachillae (Savory 1974 personal communication).

The ground leaf was pelleted with the addition of 1 per cent Tristerine and 8 per cent water.

The pellets contained 11 g/kg Mimosine (T.P.I. 1977 personal communication) and a Tannin content of 10.15 g/kg DM (Milne 1976 personal communication).

B grade leucaena. : Unseived, dry greenleaf together with pods, flower heads, rachises, rachillae and twigs smaller than 5 mm diameter (Savory 1974 personal communication).

The material was <u>not</u> ground before pelleting. The pellets were made without the addition of binder or water, and were more friable than the A grade pellets.

The grade B pellets contained 16 g/kg DM Mimosine (T.P.I. 1977 personal communication).

HAY Pretrial adjustment period; medium quality rye grass hay. Preliminary feed period and trial period ; a poor quality Cocksfoot with Red Clover hay which had been cut in 1976.

It was chopped to approximately 10 cm lengths.

Mineral and Vitamin Supplement Spencer's Hyphos C516.

Water Water was available at all times throughout the trial period.

5. Rations

Rations were calculated using the data shown in Table 3

Table 3 Data used for compilation of rations

Feed	DM %	D value* %	Metabolisable Energy MJ/Kg DM	Source
Hay	85	57	8.4	Anon 1975
Leucaena A	93	59	9.1	*In vitro* analysis
Leucaena B	93	48	7.4	*In vitro* analysis

D value Digestible Organic Matter in the Dry Matter.

Level of Feed: The rations were designed to provide the estimated Metabolisable Energy requirements for maintenance of a 30 kg liveweight sheep of 5.0 MJ/Day (see Appendix A) and are detailed in Table 4

Table 4 Planned levels of Feed

Treatment	Feed	Fresh weight g/day	Mm/Vit. g/day
T1	Hay	700[1]	6
T2	Hay Leucaena A	350 296	4
T3	Leucaena B	726	4
T4	Hay Leucaena B	350 363	4
T5	Leucaena A	592	4

* 1 Following refusal, the hay ration was reduced to 500g per day.

6. Trial procedure

Adjustment period.

The adjustment period lasted 11 days, during which time the sheep were housed in a large barn, and those sheep allocated to the leucaena diets were individually penned. For the first six days, leucaena pellets were fed at 250 g/head/day, with hay provided *ad lib*. The hay component of the diet was then gradually reduced and the leucaena component increased, such that on day 11 the sheep were receiving 400 g/head of leucaena pellets and approximately 400 g/head of hay daily.

The three sheep allocated to T1 were penned together and received *ad lib* hay during this period.

Preliminary feed period.

On day 12 the sheep were transferred to the Sheep Metabolism Unit and randomly allocated to metabolism crates where they were harnessed for collection of faeces (McDonald 1958). The crates were of the Rowett Research Institute design (Anon 1975) incorporating a urine collection shute beneath the expanded metal floor. They were sufficiently large to allow animals to lie down, but not to turn around.

The changeover from the adjustment to the experimental feed level (Table 4) was carried out over seven days, and it was at this time that the experimental hay was introduced. The sheep were maintained at the experimental feed level for a further five days before the start of the faeces and urine collection period. The ration was given in equal feeds at 0900 and 1630 h. Residues were removed each morning. Following hay refusal over several days, the level of feed was reduced to 500 g for the sheep on Treatment 1.

The digestibility trial

The sheep were weighed on day 1 and day 13 of the trial before their morning feed.

Residues were collected from day 2 to day 11 and bulked for each sheep.

Clean, weighed faeces bags (McDonald 1958) were fitted on day 3 and faeces were collected from day 3 to day 12. Daily collections were bulked for each sheep and stored in polythene bags at -20°C until the end of the trial period. After defreezing, the total faeces output per sheep was recorded and samples retained for analysis.

Urine was collected in bottles, to which had been added

100 ml of 25 per cent Sulphuric acid. The floors of the crates were also washed daily with 10 per cent Sulphuric acid to ensure complete drainage of urine into the collection bottles. Each morning, the bottles were emptied, the urine being bulked for each sheep in plastic buckets. The pH of each urine sample was maintained between 2 and 3 by the addition of Sulphuric acid as necessary. At the end of the trial, the total output per sheep was recorded and an aliquot retained for analysis.

7. Analysis of feeds and excretions.

Feeds

Dried grab samples of leucaena A and B pellets and hay, which had been collected daily throughout the trial were hammer milled to pass through a 1 mm screen, and used for analysis, unless the otherwise stated.

a) Dry matter was determined using unground material, by drying in a forced draught oven at 100°C for sixteen hours.

b) Modified Acid Detergent Fibre was determined for the hay, using the Clancy and Wilson (1966) modification of the Van Soest (1963) method.

c) Crude fibre was determined for the leucaena A and B pellets according to the Fertilizer and Feeding Stuffs Regulations (1968).

d) Crude Protein: Total nitrogen was determined by the

Kjeldahl method using selenium/potassium sulphate catalyst. Crude protein was calculated by multiplying total nitrogen by 6.25.

e) Ash was determined according to the Fertilizer and Feeding Stuffs Regulations (1968) by ignition in a muffle furnace at 500°C.

f) Gross Energy was determined in an adiabatic bomb colorimeter. (McDonald et al 1973).

g) Ether Extract was determined according to the Fertilizer and Feeding Stuffs Regulations (1968).

h) In vitro analysis was carried out on both grades of leucaena leaf and pellets by the two stage technique of Tilley and Terry (1963).

Faeces and Urine

a) Dry matter of faeces was determined by the method described for foods.

b) Nitrogen was determined on fresh material to prevent loss of volatile nitrogen by drying.

c) Ash. The determination was as described for food.

d) Gross Energy was determined in faeces as described for foods. Urine samples were dried on polythene in a vacuum dessicator over sulphuric acid (Alderman et al 1971).

RESULTS

a. Digestibility Data

b. Pathology and haematology data

Results

1. Animal health

Considerable health problems were encountered during the trial.

T2 Sheep 487 scoured badly on day 9 of the digestibility trial and it was taken off the experimental diet. As T5 was abandoned (see below) it was decided to repeat T2 (referred to as T2B) using sheep 346, 110, 220. This commenced a few days after the main trial had finished.

T2B Sheep 220 scoured on day 2 of the repeated trial. It then recovered following treatment with Kaolin. Faeces and urine were collected subsequently over 8 days.

T3 Sheep 499 went off its feed and developed a temperature of 104°F on day 4. It was taken off the trial.

T4 Sheep 206 scoured badly on day 4. Following treatment with Kaolin, faeces and urine collection re-commenced on day 7 and was continued for a full 10 days.

Sheep 244 scoured badly on day 11 and was taken off the trial. Results are reported for a 7 day collection period.

T5 This treatment was abandoned after sheep 105 and 108 had exhibited classic symptoms of minosine toxicity 7 days after being transferred to the metabolism crates. Sheep 105 died on the first day of the digestibility trial and sheep 483 after exhibiting similar symptoms, died the following day.

2. Analysis of Feed

Table 5 Analysis of hay and two grades of leucaena used in digestibility trial.

Feedstuff as fed	Dry Matter g/kg	Gross Energy KJ/g DM	Composition of Dry Matter g/kg DM				
			CP	Fibre*	Ether Extract	N Free Extract	Ash
HAY	87.3	18.1	80	428	-	-	63
LEUCAENA A	86.2	20.6	289	106	48	460?	95
LEUCAENA B	88.9	19.0	188	228	29	470?	84

* Hay = Modified acid detergent fibre (M.A.D. Fibre)
Leucaena = Crude Fibre.

The chemical composition of the feeds is shown in Table 5. Of particular interest was the high crude protein content and low fibre level of the leucaena A grade pellet.

3. Feed Digestibility

i) Hay. The hay was of rather poor quality and was not readily eaten by the sheep. Although the D value of 51 per cent was similar to published values for poor quality grass hays, the crude protein content and crude protein digestibility was much lower as shown in Table 6.

Table 6 Digestibility of Hay used in treatment T1

Sheep Nos	Digestibility Coefficient				DCP g/kgDM	ME MJ/kgDM	D %	N retent. g/day
	DM	OM	CP	GE				
233	0.52	0.53	0.32	0.49	26	7.5	50	-3.7
157	0.52	0.54	0.31	0.49	25	7.5	50	-3.0
334	0.54	0.56	0.40	0.51	32	8.0	53	-2.2
Mean	0.53 $\pm$ .012*	.54 $\pm$ .015	.34 $\pm$ .049	.5 $\pm$ .012	27	7.7	51	

* Standard deviation in all tables

Table 7 Comparison of published data on hays with results from digestibility trial

Grass hay:	Crude Protein g/kg DM	Crude Protein Digest.Coeff.	Digestible CP (g/kgDM)	Source
High digestibility	101	0.57	58	Anon 1975
Moderate "	85	0.46	39	
Low "	92	0.49	45	
Very Low "	88	0.43	38	
Hay as fed	80	0.34	27	

The maximum theoretical crude protein digestibility of the hay in this trial, was 0.63 assuming a loss of 3g metabolic faecal nitrogen per 100g of dry matter ingested (McDonald et al 1973) The crude protein content of the hay did not therefore appear to be the limiting factor in crude protein digestibility.

The benefit in this trial of using a poor quality roughage was that it conformed more generally to the type of roughage available in the tropics, with which leucaena is likely to be fed. In fact, the hay was not dissimilar in nutritive value to maize straw (Table 8), which is a common dry season feed in Africa.

Table 8 Comparison of Hay as fed with published data for Maize Straw (ANON 1975)

	Hay as fed	Maize Straw
Metabolisable Energy (MJ/kg DM)	7.7	7.3
Crude Protein (N x 6.25)(g/kg DM)	80	59
Crude Protein Digestibility Coefficient	0.34	0.34
Digestible Crude Protein (g/kg DM)	27	20
D(Digestible Organic Matter in Dry Matter)(%)	51	51

ii) Leucaena A.

Digestibility data for the leucaena A with hay treatment (T2 and T2B) are presented in Table 9 . The organic matter digestibility was very much lower than expected.

Table 9 Digestibility data for the leucaena A with hay diet (T2 & T2B)

Sheep Nos	Digestibility coefficient				DCP g/kgDM	ME MJ/kgDM	D %	N retent. g/kg
	DM	OM	CP	GE				
346	0.50	0.52	0.51	0.47	89	7.5	48	+2.1
406	0.53	0.55	0.54	0.50	94	7.9	50	+0.7
346*	0.54	0.57	0.53	0.54	93	9.0	53	+2.1
110*	0.50	0.54	0.56	0.50	98	8.0	50	+0.6
220*	0.51	0.54	0.58	0.52	101	8.4	50	+0.7
Mean	0.52±.018	0.54±.018	0.54±.027	0.51±.026	95	8.2±.57	50	

Calculations of digestibility coefficient by difference are shown in Table 10 using the hay values shown in Table 6 . The calculated values are all considerably lower than expected.

Table 10 Digestibility data for Leucanea A calculated by difference

Sheep No	Digestibility coefficients				DCP g/kgDM	ME MJ/kgDM	D %
	DM	OM	CP	GE			
346	0.46	0.49	0.56	0.44	162	7.3	44
406	0.53	0.55	0.60	0.50	173	8.1	50
346	0.55	0.61	0.59	0.58	171	10.6	55
110	0.48	0.53	0.62	0.50	179	8.4	48
220	0.49	0.54	0.66	0.54	191	9.2	49
Mean	0.50±0.037	0.54±.043	0.61±.037	0.51±.052	175	8.7±1.25	49

iii) Leucaena B.

Digestibility data for the mixed hay and leucaena B diet (Table 11); for leuceana B fed alone (Table 12); and for leucaena B determined by difference (Table 12) suggested that a depression in organic matter digestibility of seven percentage units had taken place when leucaena B was fed with hay.

Table 11 Digestibility data for Leucaena B with hay diet (T4)

Sheep No	Digestibility coefficient DM	OM	CP	GE	DCP g/kgDM	ME MJ/kgDM	D %	N Retent. g/day
135	0.42	0.42	0.44	0.37	68	5.3	40	-2.2
244	0.42	0.43	0.44	0.38	59	5.8	40	+1.6
206	0.45	0.47	0.48	0.43	65	6.5	44	+0.9
Mean	0.43 ± .017	0.44 ± .026	0.45 ± .023	0.39 ± .032	64	5.9 ± .60	41	

Table 12 Digestibility of Leucaena B when fed alone (T3) and when calculated by difference (T4)

	Sheep No	Digestibility coefficient DM	OM	CP	GE	DCP g/kgDM	ME MJ/kgDM	D %	N Retention g/day
T3	220	0.44	0.44	0.42	0.40	79	5.8	40	- 1.3
	110	0.42	0.42	0.51	0.39	96	5.7	39	+ 1.3
T4	135	0.36	0.36	0.46	0.31	86	4.1	33	
	244	0.32	0.32	0.47	0.27	88	4.0	29	
	206	0.39	0.41	0.53	0.36	100	5.5	38	

No such depression had taken place in the crude protein digestibility which has an average value of 0.48 ± .043. This would be expected in view of the much higher crude protein levels in the leucaena, compared with the hay.

Pathology and haematology data

Report on sheep 105 and 483

Both animals were allocated to the pure leucaena A diet (T5) and had been taken through the extended introductory feed period.

7 days after transfer to the Metabolism Unit, and 3 days after reaching the experimental feed level (680g of pellets/day) sheep 105 did not finish its feed. The following day it was salivating profusely and had a temperature of 105°F. It was taken off the trial and given hay and vitamins. Extensive erosion of the buccal mucosa developed. The sheep died 5 days after the onset of symptoms.

Sheep 483 started to cough up watery mucous 7 days after reaching the experimental feed level. It also had a temperature of 105°F and died the following day.

Post mortem report: sheep 105.

No.105 (P.M. 246/77)

Post-mortem examination was carried out 14 hours after death. It weighed 31.5 Kg. and showed no evidence of fat in the subcutaneous and intra-abdominal fat depots. There was general wool slip and the "clits" of supernumerary digits were shed from the hind limbs and could be easily removed from the fore limbs by traction.

The nose, buccal mucosa, tongue and posterior pharynx all appeared congested with a thick white deposit on the surface.

The retropharyngeal lymph nodes were very enlarged and congested.

The upper oesophagus was relatively normal while the lower oesophagus was thick-walled due to congestion and oedema of the submucosa while the epithelium appeared intact.

The rumen was impacted by large amounts of very dry ruminal content and the ruminal papillae appeared few in number, short and congested. (see plate I)

The reticulum and omasum also contained very dry content while the remainder of the alimentary tract contained small amounts of fluid material in apparently normal mucosa. The caecum content was soft while hard pelleted material covered by mucus was present in the proximal colon.

The lung showed a severe necrotising pneumonia with fibrinous pleurisy involving the right apical lobe, and both lungs generally appeared very congested.

Microbiological examination of the lung resulted in the isolation of a heavy mixed bacterial growth in which Pasteurella haemolytica and Corynebacterium pyogenes predominated but the bone marrow failed to demonstrate any significant bacteria.

The heart showed marked petechiation and an early fibrinous pericarditis.

The kidneys were pale in colour and soft in consistency.

The bladder appeared normal.

Other viscera not mentioned appeared normal.

No.483 (P.M. 248/77)

Examined 6-8 hours after death.

It weighed 34 Kg. There were similar findings of wool slip but in this case the "clits" were still present and could not be pulled off.

The Buccal Mucosa, tongue, hard palate, pharynx all showed similar but much more severe necrosis/erosion of the lining epithelium with severe underlying congestion. See Plates II and III.

The oesephagus throughout its length shows severe congestion of the mucosa with epithelial desquamation, this being most severe near the entrance to the rumen where the wall generally is thickened, congested and oedematous. See Plate IV.

The rumen contents are very fluid and the rumen was distended with gas. This is in direct contrast to No.105.

The reticulum and omasum and contents both showed no macroscopic abnormality.

The abomasum contains a minimal amount of food but there appears to be excess mucus production and the mucosa appeared very congested.

The small intestine especially the duodenum is very congested and the contents are blood-stained - this becomes reduced more distally with some segments contracted and empty while other segments are distended with fluid green content. The mucosa of the ileum and colon are congested - but the content is soft and unformed.

The lungs show only severe congestion.
In contrast with No. 105 there is no evidence of pneumonia or pericarditis.

The heart shows no evidence of petechiation.

The kidneys are pale, soft in consistency.

The bladder is contracted. The bladder mucosa and the urine appear normal.

Other viscera not mentioned appeared normal.

Microbiological examination of the spleen which appeared normal macroscopically yielded a heavy mixed bacterial growth in which the predominant organisms proved to be coliforms and Corynebacterium pyogenes. This is considered to be a terminal bacteremia.

Table 13 Post mortem report - organ weights (g)

	No. 105		No. 483	
Body weight	31.5 Kg.		34 Kg.	
Thyroid	1.62	1.62	2.00	1.37
Adrenal	2.35	2.93	3.28	3.58

Haematology

Blood cell count data are shown in Table 14

a. Bleeding dates. The sheep were bled on day 1 of the digestibility trial. Details of the preliminary feeding programme are given in the material and methods section. Blood samples were also taken at the end of the trial.

b. Case histories

Sheep 483 (T5) Although this sheep died within 24 hours (see post mortem report), blood cell counts appeared normal.

Sheep 108 (T5) Symptoms of mimosine toxicity, including profuse salivation, occurred 5 days before blood sampling and leucaena was withdrawn. Its temperature was 103.5°F throughout this period. 2 days post bleeding, its temperature increased to 105°F and steroids and anti-biotics were administered. The sheep recovered although it lost its fleece (Plate IX). Its blood cell count appeared normal.

Sheep 499 (T3) Following prolonged feed refusal (approximately 50%) leucaena was withdrawn from the diet on the day that it was bled. It developed a temperature of 104°F three days later and finally recovered following treatment with antibiotics. Its S.G.O.T. reading was normal.

c. Analysis of blood sampled at the end of the trial.

i) Treatment 2. Sheep 346 and 487 exhibited fleece loss. Sheep 487 was taken off trial following severe scouring (5 days before blood sampling). Its S.G.O.T. reading showed marked elevation.

Sheep 346 had an elevated white blood cell level, and sheep 220 a slightly increased red blood cell level.

ii) Treatment 3. Sheep 220 had a marked white blood cell increase, and a slightly elevated red blood cell count. It lost its fleece, but otherwise showed no ill effect from the diet.

iii) Treatment 4. Sheep 135 did not lose its fleece.

d. Conclusion

There was no consistant treatment effect on blood cell counts. Sheep 346 and 220 appeared to be combating a bacterial invasion which may have been related to the diet. Conversely, sheep 483 showed no such elevated white blood cell count, yet microbiology at post mortem showed a heavy bacterial invasion of the spleen.

Table

Blood Cell counts

DATE	SHEEP No	Treatment	Comment	RBC	PCV	Hb	MCV	WBC	NP	LC	MC	EP	SGOT
				x10^6/µl	%	g/dl	fl	x10^3/µl	%	%	%	%	iu/l
			Normal range*	9-15	27-45	9-15	28-40	5-12	10-50	40-75	0-6	0-10	20-60
25-7-77 1st day of digestibility trial -20 days after	483	100% A	Died 26-7-77	17.5	45.0	17.4	33	6.5	-	-	-	-	52.0
	108	100% A	Off trial 27-7-77	16.6	47.0	16.9	-	9.8	-	-	-	-	52.0
	499	100% B	Off trial 28-7-77	-	-	-	-	-	-	-	-	-	80.0
	233	100%Hay		15.7	35.7	17.2	26	12.0					101.0
Leucaena fed 8-8-77 End of Digestibility trial	346	50% A	Fleece loss	14.7	34.8	15.5	28	19.2	41	56	3	0	66.9
	135	50% B		PLASMA			HAEMOLYSED					-	81.3
	220	100% B	Fleece loss	16.3	38.8	15.2	31	22.5	25	69	5	1	54.3
	406	50% A		16.3	34.2	14.5	27	10.9	20	78	2	0	58.7
	487	50% A	Off trial 2-8-77	PLASMA			HAEMOLYSED						123.6
	334	100%Hay		14.2	35.5	14.5	30	11.5	35	52	2	1	81.8

RBC = Red blood cells
PCV = Pack cell volume
Hb = Haemoglobin
MCV = Mean cell volume
WBC = White blood cell
NP = Neutrophils
LC = Lymphocytes

MC = Monocytes
EP = Eosinophils
SGOT = Serum glutamine oxalo-transaminase
* Sehalm et al (1975)

Table 14

DISCUSSION AND CONCLUSION

Discussion

1) Hay

The level of feed in Treatment 1 was reduced from 610 g DM to 435 g DM/head/day (500 g Fresh weight) following feed refused. This reduction was made during the preliminary feeding stage.

The ammended level equated to 36 g DM/kg $W^{0.73}$ and provided 67 per cent of the estimated metabolisable energy requirement for maintenance. Elliot and Topps (1963) examining the relationship between voluntary intake and crude protein levels, fed sixteen foods ranging from 2.6 to 10.0 per cent crude protein to four sheep. The food which corresponded closest to the hay fed in this trial was a mixture of 67 per cent Rhodes gram (Chloris gayana) and 36 per cent Lucerne with a mean ration crude protein content of 8 per cent. They reported an increase in voluntary intake from 41 to 59 g DM/kg $W^{0.73}$ over the trial periods, indicating an adaptation of sheep to low protein diets or to the routine of management.

If the planned daily level of hay feeding (51g DM/kg $W^{0.73}$) had been continued for a sufficiently long period it is likely all sheep would have eventually consumed their feed satisfactorally.

The effect of this low level of feed on its digestibility would be nil or positive, but unlikely to be negative (Schneider and Flatt (1974).

2) Leucaena A and B pellets

The extent to which the digestibility of the leucaena based diets was affected by toxicity reactions in the sheep is difficult to assess.

Out of a total of 12 sheep fed leucaena pellets, 2 sheep died, 2 sheep were taken off trial and 3 sheep scoured at some time during the trial.

Complete fleece loss occurred in one of the two sheep on the diet T2; in both sheep on T3; and in one of the three sheep in the T4 diet.

Despite the varied reactions by sheep to leucaena (see plates V - X in Appendix) there was no correlation apparent between fleece loss and depression in diet digestibility coefficients, as shown in Table 15 .

Table 15 Individual variation in diet digestibility coefficients from treatment means

Sheep Nos.	Fleece condition	DMD	OMD	CPD	GED
346	Complete loss	0	0	-0.2	0
110	"	-0.2	0	+0.2	-0.1
220	"	-0.1	0	+0.4	+0.1
244	"	-0.1	-0.1	-0.1	-0.1
406	Full fleece	+0.1	+0.1	0	-0.1
135	"	-0.1	-0.2	-0.1	-0.2
206	"	+0.2	+0.3	+0.3	+0.4

3) Comparison of *in vitro* and *in vivo* determinations

Table 16 Leucaena digestibilities as determined *in vitro* and *in vivo*

Feed stuff	In vitro				In vivo		
	Unstandardized	Standardised					
	OMD Decimal	OMD Decimal	D %	ME MJ/kgDM	OMD Decimal	D %	ME MJ/kgDM
Leucaena A Leaf	0.60	0.63	57	8.8	-	-	-
" " Pellet	0.63	0.65	59	9.1	0.54	49	8.7
Leucaena B Leaf	0.45	0.47	43	6.7	-	-	-
" " Pellet	0.50	0.52	48	7.4	0.43	39	5.8

Note: *In vitro* values standardised with grass

In vivo values; Leucaena A by difference, Leucaena B fed as sole diet

(i) Variations in in vitro determinations

Results of the invitro determinations suggested that pelleting had a beneficial effect on digestibility with the largest increases in the grade B material (Table 16). This was somewhat surprising, as all samples were ground to the same degree of fineness before in vitro analysis was undertaken. The leucaena A material was preground before dispatch from Malawi. Therefore, it would have been ground twice before in vitro analysis. On the other hand, the B material was not ground before pelleting. The only differences therefore between the leaf and pellet would have been due to the physical effect of the pelleting. Although some fracturing of the material was inevitable, there was little frictional heat involved in the pelleting process (Pollock 1977 personal communication).

(ii) Leucaena A digestibility determined in vitro and in vivo

The in vivo D value of this material was 9 percentage units lower than the in vitro value. Although organic matter digestibility coefficients were calculated by difference, and therefore subject to considerable error, no single calculated coefficient exceeded 0.61. This should be compared with the in vitro value of 0.65. As mentioned previously, grinding would depress digestibility through the increased rate of passage of digestaon through the gut. However, the extent to which this was offset by submaintenance feeding would not be quantified.

Toxicity reactions were much more severe with the leucaena A material despite the higher mimosine content of the B material (11 g compared with 16 g mimosine per kg/DM) which is in agreement with the findings of Hegarty et al (1964). They postulated that the increased rate of passage of ground material through the gut reduced the opportunity for mimosine detoxification in the rumen. This indirect evidence of increased rate of passage of digesta supports to some extent the explanation that the reduced digestibility in vivo was due to grinding.

(iii) Leucaena B digestibility determined in vitro and in vivo

The discrepancy between the in vitro and in vivo organic matter digestibility coefficient of 7 percentage units cannot be explained, particularly as at low digestibilities, in vitro value are generally lower than those determined in vivo (Minson 1973 personal communication).

It is thought unlikely that the physical effect of the pelleting operation would have greatly affected the speed of the material through the gut, as with the leucaena A pellets. This supposition is supported by the fact that toxicity reactions were less severe with the B material despite the higher mimosine level, indicating a longer rumen retention time and increased breakdown of mimosine in the rumen.

Visually, the leucaena B material appeared to be very coarse and fibrous compared with the grade A leaf, and this was confirmed by the relatively higher crude fibre level (Table 5). However compared with published values for dried grass and legumas (Anon 1975) this crude fibre level of 228 g/kg DM would not be considered high, and the contrast is further emphasised by comparison with values for maize straw of 460 g/kg DM crude fibre and a D value of 51 per cent (Anon 1975).

4) Comparison of leucaena A and B digestibilities determined in vivo

In vitro analysis and chemical composition of the leucaena A material indicated that an organic matter digestibility coefficient of 0.65 to 0.70 could be expected. It was also thought likely that the digestibility of the hay would be unaffected or enhanced when fed with leucaena, as the increased nitrogen concentration in the rumen resulting from the legume would encourage cellulolytic activity.

A comparison of the organic matter digestibility

Table 17 Organic matter digestibility coefficients as determined and as calculated by difference

Treatment	Diet	O M D coefficient
T1	1. Hay, as determined	0.54 ± .015
T2	2. Hay with leucaena A, as determined	0.54 ± .018
	3. Leucaena A by difference (Hay OMD 0.54)	0.54 ± .043
T3	4. Leucaena B as determined	0.43 ± .014
T4	5. Hay with leucaena B as determined	0.43 ± .017
	6. Leucaena B by difference (Hay OMD 0.54)	0.36 ± .045
	7. Hay by difference (Leucaena B OMD 0.43)	0.46 ± .045

coefficients for treatments T3 and T4 (see Table 17) indicates a depression in digestibility of the mixed diet, compared with each component fed alone. This depression is of the order of 5 percentage units, if the mixed diet digestibility is calculated using the means of treatment T1 and T3. That this trend is repeated in Treatment T2 can only be conjecture, and it is tempting to calculate the organic matter digestibility of the leucaena A using the value for hay as determined in Table 17 (No.7) which produces a value of 0.65. It is possible that the activity of the cellulolytic bacteria in the rumen has, in some way been reduced by the presence of leucaena. The high nitrogen levels in the legume preclude nitrogen shortage as limiting cellulose breakdown in the rumen.

Further, any component of the leucaena directly toxic to the rumen microflora would presumably have affected the *in vitro* determinations as well.

It would be of interest in a future trial to monitor leucaena digestibility through rumen sampling of fistulated sheep to determine the exact mechanisms involved.

Conclusion

The trial to determine the *in vivo* digestibility of two grades of leucaena was initiated after *in vitro* analysis had produced digestibility coefficient which were lower than expected, and in the absence of standards with which to correct them.

The results of this trial must be considered inconclusive, in view of the health problems encountered. Nevertheless, the generally low digestibility values obtained were consistent throughout the trial, although toxicity reactions were extremely variable.

It would be of considerable value to repeat the experiment using fistulated sheep adapted over a much longer time period.

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APPENDIX

Appendix A   Calculation of the Metabolisable Energy requirements for maintenance (Anon 1975)

Level of Feed

The diets were designed to provide the estimated Metabolisable Energy requirements for maintenance of a 30kg Live weight sheep kept indoors.

Calculation of Maintenance Energy allowance

The Net Energy requirement for maintenance (E_m) was calculated from the equation:

$$E_m \text{ (MJ/day)} = 0.29\ W^{0.73}$$

The efficiency with which Metabolisable Energy is used for maintenance (K_m) is 0.70, and the maintenance Metabolisable Energy requirement is therefore:

$$\frac{E_m}{0.70} \quad 1.43\ E_m$$

For a 30 kg Live weight sheep with a Metabolic Body Weight ($W^{0.73}$) of 12 kg the Metabolisable Energy requirement for maintenance (M_m) is:

$$M_m = 0.29 \times 12 \times 1.43$$

$$= \underline{\underline{5.0 \text{ MJ/day}}}$$

Appendix B

Liveweight changes during Digestibility Trials

Treatment	Sheep Nos	Liveweight (kg)		Change (kg)	Comments
		Day 1	Day 13		
T1	233	29.5	29.0	- 0.5	
	157	29.0	27.5	- 1.5	
	334	31.5	31.5	0	
T2	346	31.0	27.5	- 3.5	Complete fleece loss between weighings
	406	30.0	29.5	- 0.5	
T3	220	32.0	30.5	- 1.5	
	110	27.5	26.0	- 1.5	
T4	135	27.5	26.0	- 1.5	Complete fleece loss between weighings
	244	32.0	27.5	- 4.5	
	206	32.0	32.5	+ 0.5	
T2B	346	28.0	27.5	- 0.5	
	110	27.5	26.0	- 1.5	
	220	31.5	29.5	- 2.0	

Appendix C

Health record

Treatment No	SHEEP Nos	Remarks	DAYS ON DIGESTIBILITY TRIAL
T1	233	)	10
	157	) Full fleece	10
	334	)	10
T2	346	Fleece loss	10
	406	Full fleece	10
T2B	346		10
	110		10
	220	Scoured on day 9 of trial	8
T3	220	Fleece loss	10
	110	Fleece loss	10
T4	135	Full fleece	10
	244	Scoured on day 8 of trial. Fleece loss	7
	206	Scoured at start of trial - trial extended. Full Fleece	10

ANALYSIS OF HAY RESIDUE - SHEEP 135

DM	Analysis of DM(g/kg)		Gross Energy
(g)	CP	Ash	KJ/g DM
1454	74	96	18.14

Appendix

DRY MATTER DIGESTIBILITY

T	SHEEP	HAY INTAKE (g)			LEUCAENA A INTAKE(g)			LEUCAENA B INTAKE(g)			FAECES OUTPUT (g)			DRY MATTER DIGESTIBILITY			
																LEUCAENA*	
Nos	No Nos.	F.Wt	DM %	D.M.	F.Wt	DM %	D.M.	F.Wt	DM %	D.M.	F.Wt	DM %	D.M.	Diet	Hay	A	B
T1	233	4986.8	87.3	4353.4							6912.0	30.2	2087.4	0.52			
	157	5000.0	"	4365.0							7808.5	26.6	2077.0	0.52			
	334	5000.0	"	4365.0							5455.0	36.7	2002.0	0.54			
	MEAN													0.53			
T2	346	3500.0	"	3055.5	2960.0	86.2	2551.5				6806.5	41.3	2811.1	0.50	0.53	0.46	
	406	3500.0	"	3055.5	2960.0	"	2551.5				6132.0	43.1	2642.9	0.53		0.53	
T2B	346	3500.0	"	3055.5	2960.0	"	2551.5				6347.5	40.7	2583.4	0.54	"	0.55	
	110	3500.0	"	3055.5	2960.0	"	2551.5				5735.5	48.4	2776.0	0.50	"	0.48	
	220	2800.0	"	2444.4	2368.0	"	2041.2				5107.5	42.9	2191.1	0.51	"	0.49	
	MEAN													0.52		0.50	
T3	220							7228.5	88.9	6426.1	7736.0	46.9	3628.1	0.44			
	110							7219.5	"	6418.1	6837.0	54.1	3698.8	0.42			
	MEAN													0.43			
T4	135	1834.0	"	1601.1				3640.0	"	3236.0	6453.5	43.8	2826.6	0.42	0.53		0.36
	244	2450.0	"	2138.9				2548.0	"	2265.1	5664.5	44.8	2537.7	0.42	"		0.32
	206	3500.0	"	3055.5				3640.0	"	3236.0	7732.5	44.4	3433.2	0.45	"		0.39
	MEAN													0.43			0.36

* Leucaena digestibility calculated by difference.

Appendix D.

DRY MATTER DIGESTIBILITY

Appendix

ORGANIC MATTER DIGESTIBILITY

T Nos	SHEEP Nos	HAY INTAKE (g) D.M.	HAY INTAKE (g) %ASH	HAY INTAKE (g) O.M.	LEUCAENA A INTAKE(g) D.M.	LEUCAENA A INTAKE(g) %ASH	LEUCAENA A INTAKE(g) O.M.	LEUCAENA B INTAKE(g) D.M.	LEUCAENA B INTAKE(g) %ASH	LEUCAENA B INTAKE(g) O.M.	FAECES OUTPUT(g) D.M.	FAECES OUTPUT(g) %ASH	FAECES OUTPUT(g) O.M.	ORGANIC MATTER DIGESTIBILITY DIET	ORGANIC MATTER DIGESTIBILITY HAY	LEUCAENA * A	LEUCAENA * B
T1	233	4353.4	6.33	4077.8							2087.4	9.14	1896.6	0.53			
	157	4365.0	"	4088.7							2077.0	8.76	1895.1	0.54			
	334	4365.0	"	4088.7							2002.0	10.33	1795.2	0.56			
	MEAN													0.54			
T2	346	3055.5	"	2862.0	2551.5	9.52	2308.6				2811.1	11.43	2489.8	0.52	0.54	0.49	
	406	3055.5	"	2862.0	2551.5	"	2308.6				2642.9	11.21	2346.6	0.55	"	0.55	
T2B	346	3055.5	"	2862.0	2551.5	"	2308.6				2583.4	14.91	2198.2	0.57	"	0.61	
	110	3055.5	"	2862.0	2551.5	"	2308.6				2776.0	13.81	2392.6	0.54	"	0.53	
	220	2444.4	"	2289.8	2041.2	"	1846.9				2191.1	13.84	1887.9	0.54	"	0.54	
	MEAN													0.54		0.54	
T3	220							6426.1	8.42	5885.0	3628.1	9.24	3292.9	0.44			
	110							6418.1	"	5877.7	3698.8	8.58	3381.4	0.42			
	MEAN													0.43			
T4	135	1601.1	3.35	1547.4				3236.0	"	2963.5	2826.6	8.17	2595.7	0.42	0.54		0.36
2	244	2138.9	6.33	2003.5				2265.1	"	2074.4	2537.7	8.18	2330.1	0.43	"		0.32
	206	3055.5	"	2862.0				3236.0	"	2963.5	3433.2	10.64	3067.9	0.47	"		0.41
	MEAN													0.44			0.36

* Leucaena digestibility calculated by difference

Appendix D

ORGANIC MATTER DIGESTIBILITY

Appendix

CRUDE PROTEIN DIGESTIBILITY

T	SHEEP	HAY INTAKE (g)			LEUCAENA A INTAKE(g)			LEUCAENA B INTAKE(g)			FAECES OUTPUT(g)			CRUDE PROTEIN DIGESTIBILITY			
																LEUCAENA *	
Nos	Nos	D.M.	% CP (Nx6.25)	C.P.	D.M.	% CP	C.P.	D.M.	% CP	C.P.	Fresh WEIGHT	% CP	C.P.	Diet	HAY	A	B
T1	233	4353.4	7.96	346.5							6912.0	3.40	235.0	0.32			
	157	4365.0	"	347.5							7808.5	3.06	238.9	0.31			
	334	4365.0	"	347.5							5455.0	3.84	209.5	0.40			
	MEAN													0.34			
T2	346	3055.5	"	243.2	2551.5	28.86	736.4				6806.5	7.06	480.5	0.51	0.34	0.56	
	406	3055.5	"	243.2	2551.5	"	736.4				6132.0	7.38	452.5	0.54	"	0.60	
T2B	346	3055.5	"	243.2	2551.5	"	736.4				6347.5	7.31	464.0	0.53	"	0.59	
	110	3055.5	"	243.2	2551.5	"	736.4				5735.5	7.59	435.3	0.56	"	0.62	
	220	2444.4	"	194.6	2041.2	"	589.1				5107.5	6.38	325.9	0.58	"	0.66	
	MEAN													0.54		0.61	
T3	220							6426.1	18.79	1207.5	7736.0	9.06	700.9	0.42			
	110							6418.1	"	1206.0	6837.0	8.63	590.0	0.51			
	MEAN													0.47			
T4	135	1601.1	8.47	135.6				3236.0	"	608.0	6453.5	6.43	415.0	0.44	0.34		0.46
	244	2138.9	7.96	170.3				2265.1	"	425.6	5664.5	5.93	335.9	0.44	"		0.47
	206	3055.5	7.96	243.2				3236.0	"	608.0	7732.5	5.72	442.3	0.48	"		0.53
	MEAN													0.45			0.49

* Leucaena digestibility calculated by difference

Appendix II

CRUDE PROTEIN DIGESTIBILITY

Appendix

GROSS ENERGY DIGESTIBILITY

T	SHEEP	HAY INTAKE (KJ)			LEUCAENA A INTAKE(KJ)			LEUCAENA B INTAKE(KJ)			FAECES OUTPUT(KJ)			GROSS ENERGY DIGESTIBILITY		LEUCAENA*	
Nos	Nos	DM.(g)	GE KJ/gDM	GE	DM g	GE KJ/gDM	GE	DM.(g)	GE KJ/gDM	GE	DM.(g)	GE KJ/gDM	GE	Diet	Hay	A	B
T1	233	4353.4	18.067	78653							2087.4	19.366	40425	0.49			
	157	4365.0	"	78862							2077.0	19.362	40215	0.49			
	334	4365.0	"	78862							2002.0	19.198	38434	0.51			
	MEAN													0.50			
T2	346	3055.5	"	55204	2551.5	20.617	52604				2811.1	20.343	57186	0.47	0.50	0.44	
	406	3055.5	"		2551.5	"	52604				2642.9	20.477	54119	0.50	"	0.50	
T2B	346	3055.5	"		2551.5	"	52604				2583.4	19.212	49632	0.54	"	0.58	
	110	3055.5	"		2551.5	"	52604				2776.0	19.500	54132	0.50	"	0.50	
	220	2444.4	"	44163	2041.2	"	42083				2191.1	18.848	41298	0.52	"	0.54	
	MEAN													0.51		0.51	
T3	220							6426.1	18.974	121929	3628.1	20.133	73045	0.40			
	110							6418.1	"	121777	3698.8	20.161	74572	0.39			
	MEAN													0.40			
T4	135	1601.1		28927				3236.0	"	61400	2826.6	20.140	56928	0.37	0.50		0.31
	244	2138.9	"	38644				2265.1	"	42978	2537.7	19.931	50579	0.38	"		0.27
	206	3055.5	"	55204				3236.0	"	61400	3433.2	19.491	66917	0.43	"		0.36
	MEAN													0.39			0.31

* Leucaena digestibility calculated by difference

Appendix B

GROSS ENERGY DIGESTIBILITY

METABOLISABLE ENERGY

T Nos	SHEEP Nos	Feed intake DM(g)	Feed intake GE(KJ)	OUTPUT Faeces(KJ)	Urine g	Urine KJ/g(w/W)	Urine KJ	Methane(KJ) [1]	ME KJ/g DM Diet	Hay	Leucaena * A	Leucaena * B
T1	233	4353.4	78653	40425	8712	0.3099	2700	2910	7.5			
	157	4365.0	78862	40215	13602	0Q.2227	3029	2918	7.5			
	334	4365.0	78862	38434	16706	0.1512	2526	2918	8.0			
	MEAN								7.7			
T2	346	5607.0	107808	57186	6830	0.6853	4681	3988	7.5	7.7	7.3	
	406	5607.0	107808	54119	6652	0.7727	5140	3990	7.9	"	8.1	
T2B	346	5607.0	107808	49632	5800	0.6545	3796	3993	9.0	"	10.6	
	110	5607.0	107808	54132	5600	0.8300	4648	3990	8.0	"	8.4	
	220	4485.6	86246	41298	4600	0.9004	4142	3193	8.4	"	9.2	
	MEAN								8.2		8.7	
T3	220	6426.1	121929	73045	10524	0.6547	6890	4506	5.8			
	110	6418.1	121777	74572	8758	0.6782	5940	4499	5.7			
	MEAN								5.8			
T4	135	4837.1	90327	56928	16628	0.2699	4488	3332	5.3	7.7		4.1
	244	4404.0	81622	50579	4388	0.5621	2466	3015	5.8	"		4.0
	206	6291.5	116604	66917	12130	0.3468	4207	4310	6.5	"		5.5
	MEAN								5.9			4.5

1. Methane estimation (Blaxter & Clapperton 1965)

Per 100 KJ of food fed

Methane (KJ) = 3.67+0.062xDE

DE = Gross energy digestibility

* Leucaena digestibility calculated by differences

Appendix E

METABOLISABLE ENERGY

Appendix

NITROGEN RETENTION

T Nos	Sheep Nos	NITROGEN INTAKE: HAY DM (g)	HAY % N	HAY N(g)	LEUCAENA A DM (g)	LEUCAENA A % N	LEUCAENA A N (g)	LEUCAENA B DM (g)	LEUCAENA B % N	LEUCAENA B N (g)	NITROGEN OUTPUT: FAECES F.Wt(g)	FAECES % N	FAECES N (g)	URINE Wt(g)	URINE % N	URINE N g	Average Daily N retent. (g)
T1	233	4352.5	1.274	55.5							6912.0	0.544	37.6	8712.0	0.630	54.9	-3.7
	157	4364.0	1.274	55.6							7808.5	0.490	38.3	13602.0	0.350	47.6	-3.0
	334	4364.0	1.274	55.6							5455.0	0.614	33.5	16706.0	0.262	43.8	-2.2
T2	346	3054.8	1.274	38.9	2551.8	4.617	117.8				6806.5	1.1291	76.8	6830.0	0.866	59.2	+2.1
	406	3054.8	1.274	38.9	2551.8	4.617	117.8				6132.0	1.180	72.4	6652.0	1.166	77.6	+0.7
T2B	346	3054.8	1.274	38.9	2551.8	4.617	117.8				6347.5	1.17	74.3	5800.0	1.050	60.9	+2.1
	110	3054.8	1.274	38.9	2551.8	4.617	117.8				5735.5	1.215	69.7	5600.0	1.448	81.1	+0.6
	220	2443.8	1.274	31.1	2041.5	4.617	94.3				5107.5	1.02	52.1	4600.0	1.476	67.9	+0.7
T3	220							6427.0	3.007	193.3	7736.0	1.579	122.2	10524.0	0.794	83.6	-1.3
	110							6418.9	3.007	193.0	6837.0	1.408	96.3	8758.0	0.961	84.2	+1.3
T4	135	1600.8	1.355	21.7				3236.3	3.007	97.3	6453.5	1.029	66.4	16628.0	0.448	74.5	-2.2
	244	2138.4	1.274	27.2				2265.4	3.007	68.1	5664.5	0.949	53.8	4388.0	0.683	30.0	+1.6
	206	3054.8	1.274	38.9				3236.3	3.007	97.3	7732.5	0.915	70.8	12130.0	0.469	56.9	+0.9

Note: Sheep 244 (T4) 7 day feed
Sheep 220 (T2B) 8 day feed

Appendix D

NITROGEN BALANCE

Plate I Rumen impaction [Sheep 105]

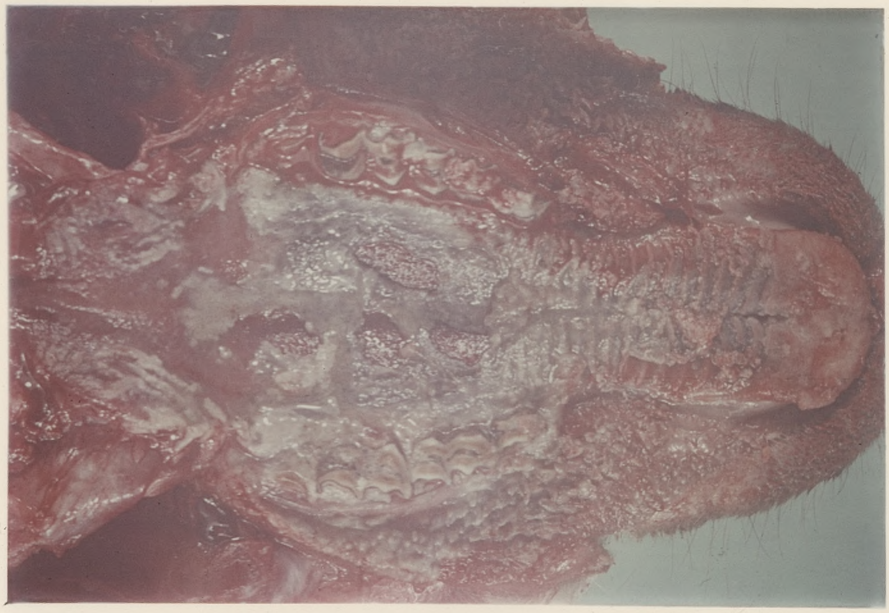

Plate II Stomatitis [Sheep 483]

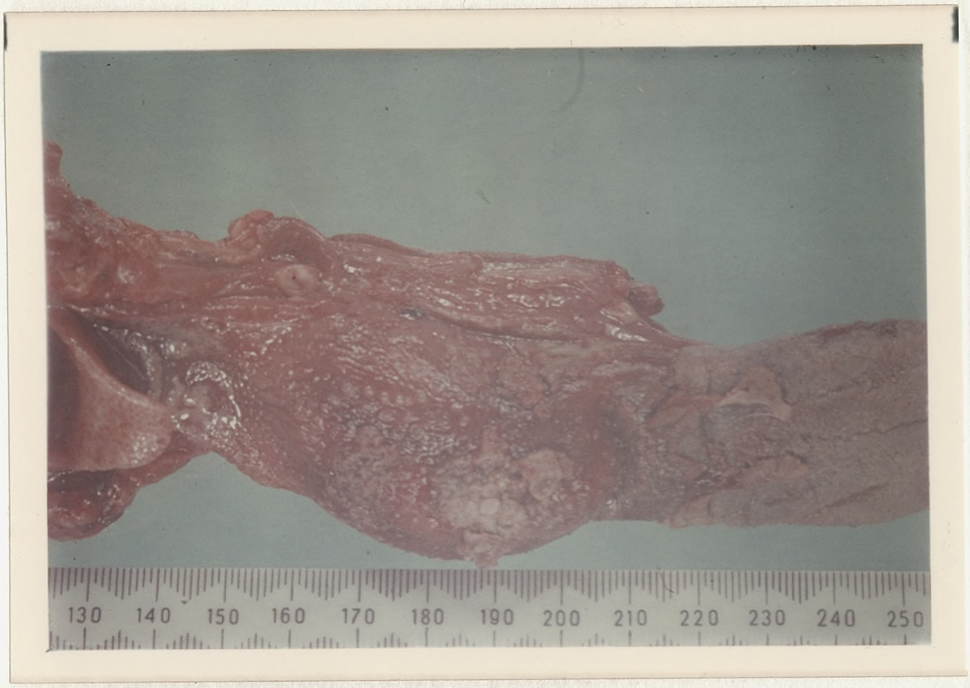

Plate III   <u>Stomatitis</u>   [Sheep 483]

Plate IV   <u>Oesophagitis</u>   [Sheep 483]

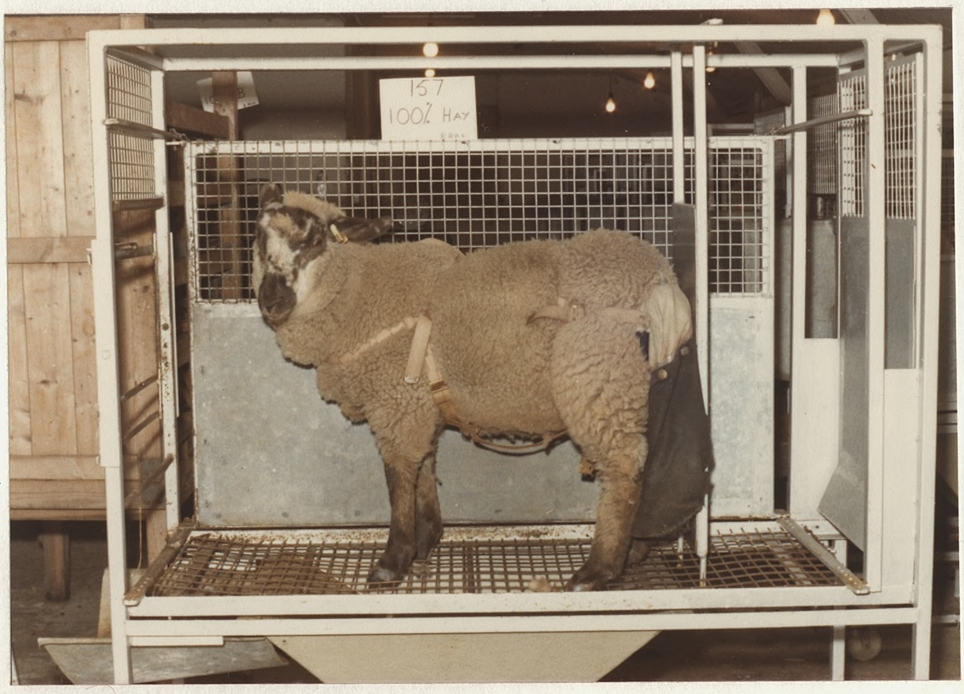

Plate V HAY ONLY T1 [Sheep 157]

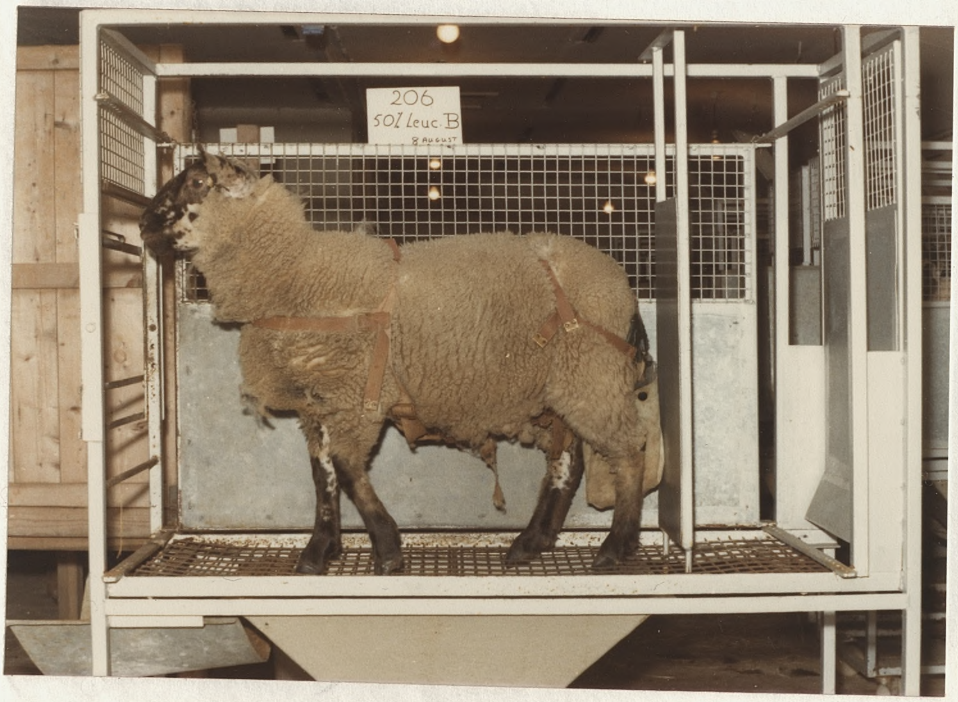

Plate VI HAY WITH LEUCAENA B T4 [Sheep 206]

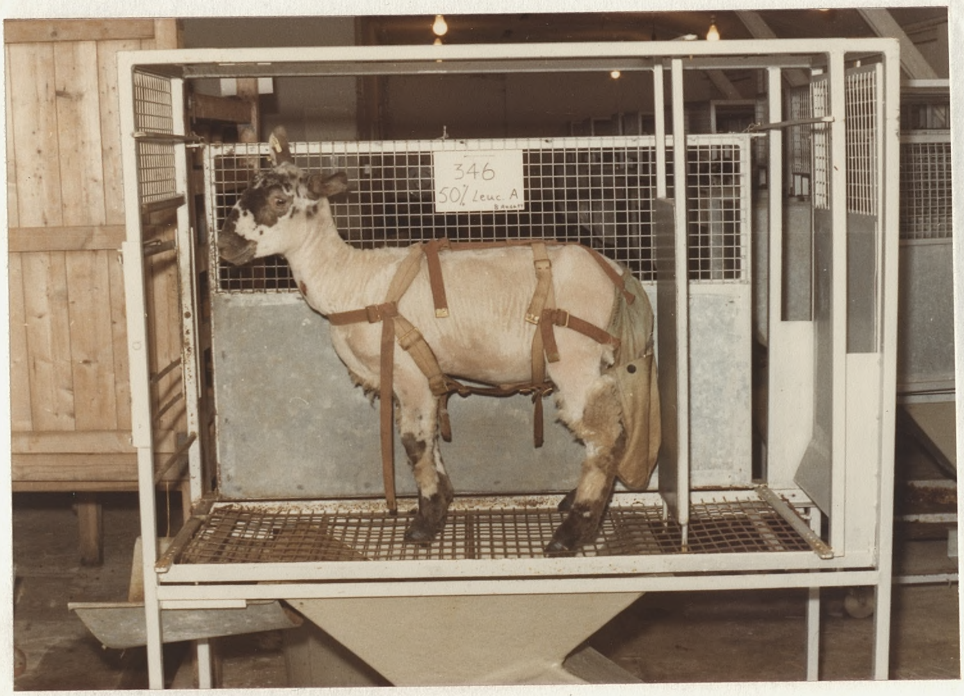

Plate VII   Hay with Leucaena A   T2   [Sheep 346]

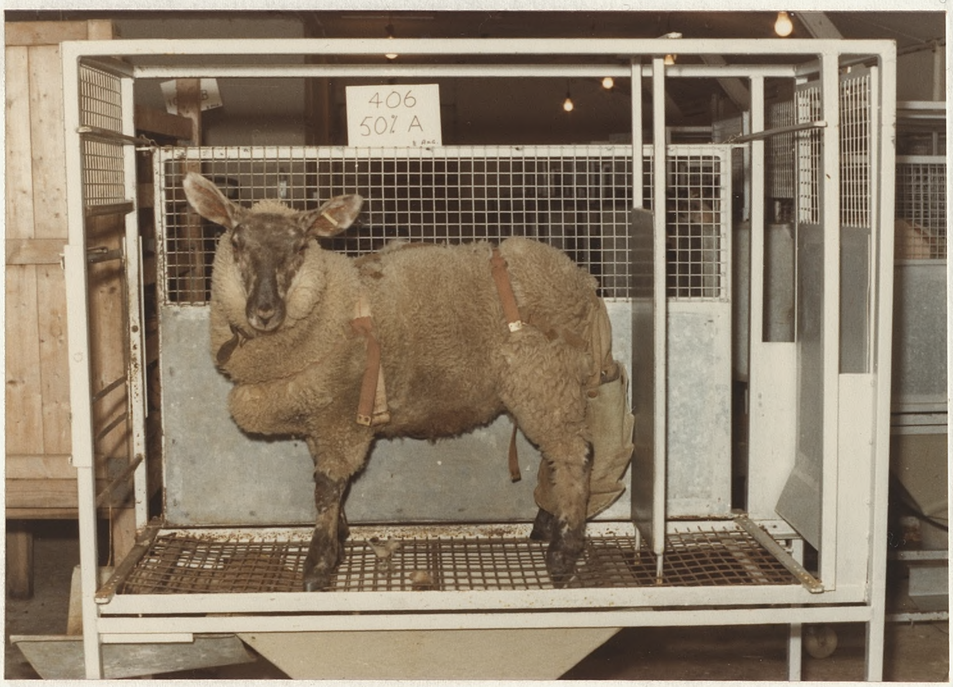

Plate VIII   Hay with Leucaena A   T2   [Sheep 406]

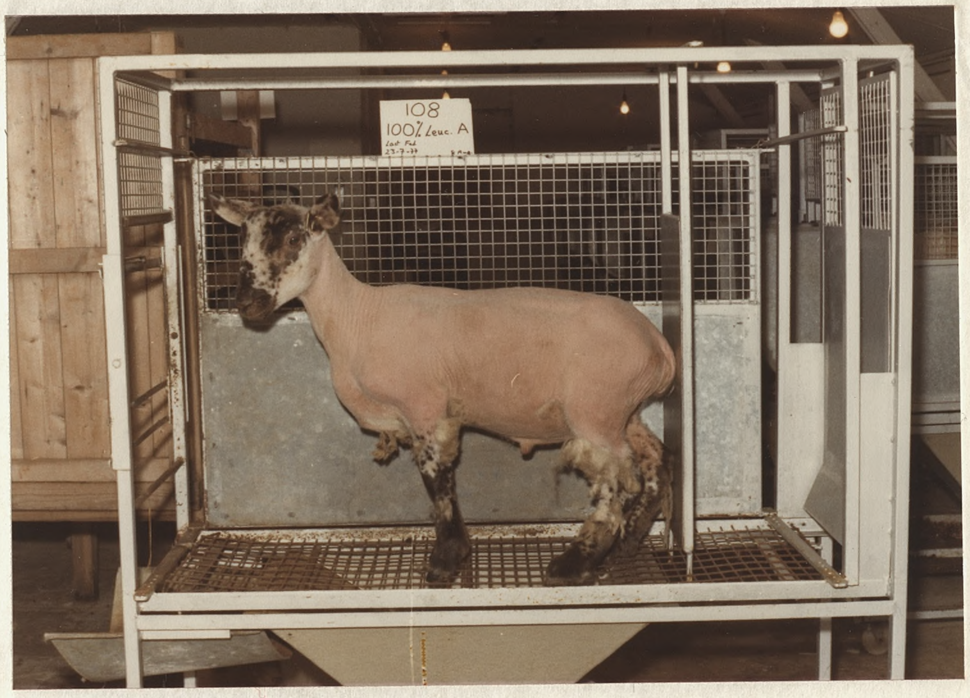

Plate IX Leucaena A only T5 /Sheep 108/

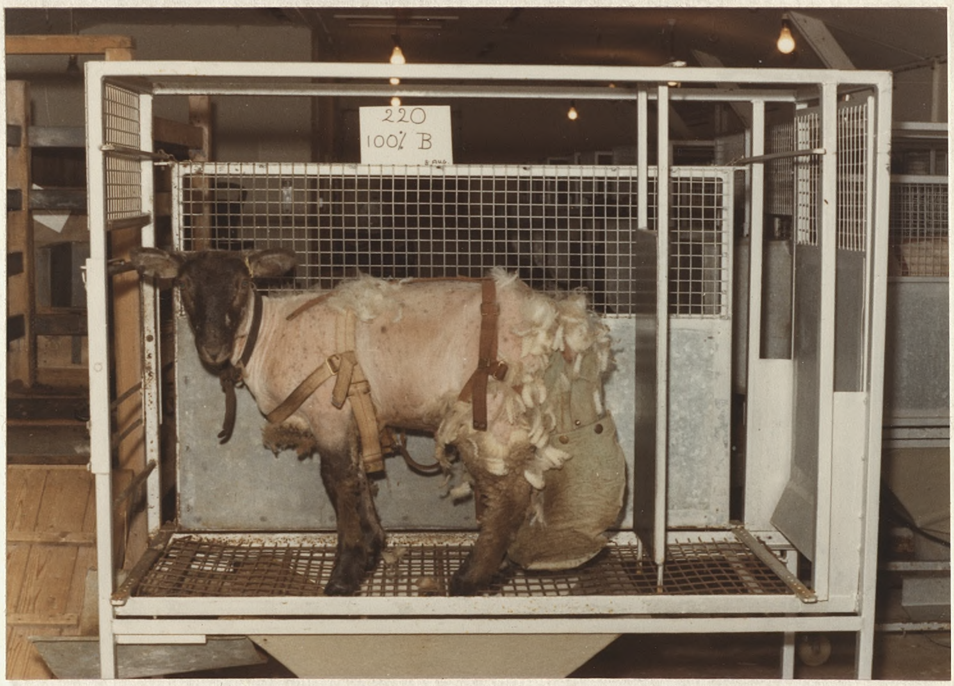

Plate X Leucaena B only T3 /Sheep 220/