MICROBIAL DEGRADATION OF MIMOSINE IN GOATS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **MICROBIAL DEGRADATION OF MIMOSINE IN GOATS** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **MICROBIAL DEGRADATION OF MIMOSINE IN GOATS** is a record of research work done independently by Sri. P. Prabaharan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Dedicated to my parents

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Introduction

INTRODUCTION

Livestock production in many countries of the world especially in those of the tropics and subtropics, is limited by a deficiency of protein, the key nutrient which decides the biological as well as economic efficiency of animal system as a whole. Protein deficiency is particularly marked in areas with a long dry season when green fodder is limited or totally absent. Tree fodder can play an important role in mitigating the fodder shortage in the dietary of animals.

The use of legume fodders during the dry season has resulted in a dramatic increase in animal production in the tropics (Stobbs, 1972) which, in turn, encouraged the more wide spread adoption of this technology. A number of legumes are now available for use in the tropical and sub-tropical areas of the world (Hutton 1970, 1974) and among these Leucaena leucocephala (subabul) has been given great prominance in a publication in the National Academy of Sciences (NAS, 1977).

Leucaena is an arbarescent legume shrub or tree belonging to the mimosiaceae. This legume is known variously as "Jumbie Bean", "Jumbey" or "Jimbey" in the Bahamas, "Lamtoro" in Indonesia and "Koa Haole" in Hawaii. (Owen, 1958). The species L. leucocephala is represented by a number of plant forms (Jones, 1979). The most wide spread in many tropical areas is a shrubby free seeding form known as "Hawaiian" leucaena, which tends to be woody and low yielding. More productive, multi branched, semi erect, medium height forms and those that flower later than the Hawaiian, are represented by the cultivars, Peru and Cunningham. The tall, erect, sparsely branched forms, which may also be grown as forest trees, are represented by the "giant" cultivars K-8 and K-28. All are palatable to livestock and regrow rapidly after cutting or grazing.

Leucaena grows well in a wide range of soils with the marked exception of very acid soils (with low base status) and water logged soils, and is particularly well adapted to calcareous clay soils (Oakes, 1968; Gray, 1968 and Hill, 1971). Once established, it requires little more field care than does napier grass. It's low moisture requirements and the ability to recover after drought, extend its usefulness to lands not suited to other legumes. Little or no fertilizer is required in its cultivation (Tahahashi and Ripperton, 1949 and Singh and Mudgal, 1967).

The species will grow anywhere in the tropics and sub tropics within an annual rainfall range of 500 to 3000 mm. Its rhizobium requirements are highly specific and all seeds must be inoculated. While able to grow in frosty conditions, its yield is greatly reduced at low temperatures, at high altitudes and in dry conditions (Jones, 1979). According to him, in the wet tropics, yield of 20 tonnes dry matter/ha/year have been obtained with crude protein yields in excess of 3 tonnes/ha. Relwani and Rangnekar (1979) observed that, it gives about 100 tonnes of green fodder or 30 to 35 tonnes of dry matter containing 6.5 to 7.5 tonnes of crude protein per hectare. These are much higher than for most other tropical legumes and are equivalent to nitrogen fixation rates of up to 500 kg N/ha/year. At the other end of the scale, yields of 5 tonnes/ha/year can be expected under cooler or drier conditions. Singh and Mudgal (1967) reported yields of 375 quintals of nutritious fodder per acre per year in about 4 cuttings besides producing about 13 quintals of seed per acre which can be used as a concentrate feed after qrinding.

The original habitat of leucaena is Mexico but has been introduced in many parts of the world. There has been a lot of interest in the higher rainfall tropical and sub tropical regions of Australia in the use of this fodder (Hutton and Gray, 1959 and Hutton and Bonner, 1960). It was introduced in India in the year 1962 at the National Dairy Researh Institute Farm, Karnal, with the seed obtained from Hawaii, where in the performance with dairy cattle had been very good. Since then, several studies have been carried out regarding its nutritive value and based on the promising results obtained in India and abroad, attempts are underway in various parts of India to introduce subabul in the national grass lands with a view to improve the forage quality and to provide enough nutritious green fodder to various categories of livestock, especially during the lean periods of the year.

In the meantime, there has been, however, several reports from various parts of the world, regarding some adverse effects of feeding subabul on account of the toxic principle mimosine present in it (Hegarty et al., 1964; Hylin, 1964; Jones and Megarrity, 1983 and Allison et al., 1990). The contrasting responses of goats fed 100 per cent subabul based ration in Australia and Hawaii, have been reported by Jones and Megarrity (1983). Goats in Australia showed signs of mimosine toxicity while those in Hawaii fed on a similar diet given at a higher level of intake of mimosine showed no symptoms of toxicity, the difference being attributed to the microbial degradation of mimosine in the rumen of animals in Hawaii. The extent of degradation of mimosine in the rumen varies with the species of animals, geographic area and composition of diet (Jones and Megarrity, 1983).

No systematic work, has thus far been carried out on the rumen microbial degradation of mimosine by Indian goats and particularly so with those in Kerala. Before popularising large scale cultivation and feeding of subabul, it is essential to find out the extent of rumen microbial degradation of mimosine, if any, by goats so as to confirm the possibility of large scale feeding of goats on subabul especially during summer season when there used to be acute shortage of green forage.

Review of Literature

REVIEW OF LITERATURE

2.1. Chemical composition and nutritive value of subabul

2.1.1. Chemical composition

Although a shrub, leucaena is very palatable to livestock. Regrowing shoots, 0.5 to 1 m long are usually composed of 75-80 per cent of highly nutritious leaf. The young green stems up to 5-6 mm diameter are also eaten by animals. Thicker stems are far less palatable and nutritious than the leaf or young stems.

The leaves, young stems, flowers and pods of leucaena are all excellent sources of protein and minerals. The chemical composition of different parts of the plant seems to vary probably due to climatic or edaphic factors. According to Singh and Mudgal (1967), the crude protein content varied from 18.9 to 27.57 per cent, ether extract from 2.59 to 5.83 per cent, crude fibre from 10.16 to 17.23 per cent, calcium from 0.8 to 1.99 per cent and phosphorus from 0.16 to 0.37 per cent on dry matter basis in the case of leucaena glauca, analysed during each month starting from October to September, even though, there was no correlation observed between the nutrients. They found that the crude protein contents were the highest during July when fat and minerals were the least. Crude protein content to the extent of 35.1 per cent was reported by Hutton and Bonner (1960). They found the lowest protein level of 20.1 per cent during the month of June when the growth was slow. James (1978) reported that L. leucocephala fodder contains 32.5 per cent dry matter, 18.2 per cent crude protein, 6.2 per cent ether extract, 13.2 per cent crude fibre, 54 per cent nitrogen free extract, 8.4 per cent ash, 2.2 per cent calcium, 0.25 per cent phosphorus and 0.32 per cent magnesium on dry matter basis. According to Adeneye (1979), the edible parts of leucaena contains dry matter ranging from 19.2 to 86.4 per cent, crude protein 8.2 to 47.4 per cent, ether extract 0.3 to 13.8 per cent, crude fibre 3.4 to 35.8 per cent,ash 3.8 to 9.3 per cent and nitrogen free extract 29.9 to 66.8 per cent on dry matter basis. Bhaskar et al. (1987) reported 30.44 per cent crude protein in L. leucocephala Hawaiian Giant K-8 compared to 22.86 per cent and 21.45 per cent respectively in the case of L. glauca (white popiane green) and L. leucocephala (Lam) de-Wit green leaf. From a comparative study involving 12 cultivars of subabul, Akbar and Gupta (1985) reported 25 per cent or more of crude protein in respect of 'Cunning ham', 'Peru - I', and 'Peru II'. According to them, the crude protein contents in the cultivars varied from 19 to 26 per cent and were in the same range as that of cultivated fodders (Gupta and Pradhan, 1975 and Singh et al., 1976). Similar values were also reported by Kearl (1982); Jones (1979); Satya sitaraman (1980); Prasad et al. (1983); Vahidulla (1984)., Akbar and Gupta (1985); Gupta et al. (1986); Hongo et al. (1987); Kumar et al. (1987); Sudhakar and Rama Rao (1987); Gupta et al. (1988); Gupta et al. (1989) and Sunaria and Vidya Sagar (1989).

The proximate analysis of various plant parts of the variety "K-8" carried out by Akbar and Gupta (1985) showed that the seeds, leaves and pods are richer in protein, the values being considerably higher in the case of seeds. According to them, seeds contain the highest amount of crude fat compared to other edible parts of the plant as also reported by Adeneye (1979). Leucaena seeds are richer than the cluster bean seeds in ether extract, at par in crude protein and total minerals, but lower in NFE contents (Gupta et al., 1978). According to Jones (1979), leaf material of leucaena compares favourably with alfalfa or lucerne (Medicago sativa) leaf material in terms of protein and minerals. He also pointed out that these two species are very similar in respect of their amino acid composition except for the higher isoleucine content of 563 mg/g N. in the former as compared to 290 mg/g N. in the latter.

Unlike other legumes, subabul fodder is very high in tannins (Jones,1979). According to him, while alfalfa leaf meal contains 0.13 mg tannin per g, subabul leaf meal contains 10.15 mg/g. Akbar and Gupta (1985) reported that the tannin contents in the 12 different cultivars they studied, varied from 7.33 mg/g ('K-8') to 19.66 mg/g (Dehra Dun). The presence of significant amounts of tannins in leucaena has important nutritional implications as far as the ruminant is concerned and unlike with lucerne, no cases of bloat have been noted in cattle grazing on leucaena (Jones, 1979). These tannins may also have an important role in the protection of protein from degradation in the rumen and therefore, in making it more available in the small intestine.

Leucaena leaf material is an excellent source of ß-carotene, which could be a valuable characteristic, particularly during the dry season when leucaena is able to retain green leaf better than many other pasture species. According to Jones (1979), leucaena leaf contains 536.0 mg carotene/kg as compared to 253.0 mg/kg of alfalfa leaf.

The total ash and mineral contents in leucaena seem to vary considerably (Jones, 1979; Akbar and Gupta, 1985, and Rangnekar and Joshi, 1980-81). According to Jones (1979), the calcium concentration in leucaena appears to vary considerably, depending on the location. For example, under Australian conditions, on a variety of soils, the calcium concentration rarely exceeds 1.0 per cent on dry matter basis whereas in India and Malawi, values of more than 2.0 per cent calcium are reported in leucaena leaf. From a comparative study involving 12 cultivars, Akbar and Gupta (1985) reported that the concentration of calcium in leucaena ranged from 1.7 per cent in "Dehra Dun" to 2.9 per cent in "K-8", while that of phosphorus ranged from 0.14 per cent in "Australian" and "Hawaiian common" to 0.35 per cent in "K-8" with calcium : phosphorus ratio ranging between 7:1 and 15:1 as against calcium : phosphorus ratios of 13:1, 16:1 and 22:1 respectively reported by D'Mello and Thomas (1977), Upadhyay _et al._,(1974) and Rosas _et al._, (1981). From a study involving 12 cultivars of subabul, Akbar and Gupta (1985) concluded that the concentration of calcium in all samples analysed compared well with the N.R.C (1971) recommendations of 0.2 to 0.25 per cent for cattle while that of phosphorus is not adequate to meet the requirement, in "Australian common" and "Hawaiian common" varieties. All the 12 cultivars could meet the N.R.C requirement (N.R.C 1971) for sulphur and potassium but were poor in sodium. According to Jones (1979), the leaves, young stems, flowers and pods are all excellent sources of minerals except sodium which is low in all plant parts. Iodine content also has been found to be low in leucaena, varying from 33 to 90 µg per kg. and this compares with the requirement by ruminants of approximately 800 µg per kg (0.8 ppm) (Agricultural Research Council, 1965). The zinc contents ranging from 30 to 170 ppm reported by Akbar and Gupta, (1985) for 12 cultivars of subabul are within the range reported by Rengnekar and Joshi (1980-81). According to them, all the cultivars can meet the N.R.C (1971) requirement of iron (100 ppm), copper (10 ppm), manganse (20 ppm) and zinc (20 ppm) for cattle.

The mineral content of different plant parts of subabul "(K-8") vary widely (Akbar and Gupta, 1985). They observed that calcium content is highest in leaves (3.0%) and lowest in seeds (0.85%) while phosphorus content is highest in seeds (0.6%) and lowest in dry pods (0.1%). The values for Fe, Cu, Mn, and Zn vary from 250 ppm (seeds and green pods) to 525 ppm (mature leaves), 25 ppm (branches) to 45 ppm (mature leaves), 38 ppm (seeds, dry pods and green pods) to 115 ppm (mature leaves) and 10 ppm (dry pods) to 97 ppm (green pods) respectively.

2.1.2. Nutritive value of subabul for cattle and Buffalo

There are many reports on the digestibility of leucaena in ruminants. Dry matter digestibilities ranging from 50 to 71 per cent are reported in the literature (Singh and Mudgal, 1967; Upadhyay et al., 1974; Joshi and Upadhyay, 1976 and Bhaskar et al., 1987). The lower digestibility values were obtained when leucaena was fed in a mixture and the digestibility calculated by difference and the higher value of 71 per cent was obtained using goats fed on leucaena alone. The value of 71 per cent accords with values for the digestibility if leaves and young shoots of leucaena are fed as sole diet to cattle and goat (Jones, 1979).

Singh and Mudgal (1967) recorded 3.58 per cent DCP and 16.15 per cent TDN on fresh basis as green leaf when fed to male calves.

Pathak et al., (1977) reported average digestibility coefficients of 71.4, 78.0, 47.6, 57.7 and 81.1 respectively for dry matter, crude protein, ether extract, crude fibre and nitrogen free extract with DCP and TDN values of 16.73 and 70.22 per cent respectively, when L. leucocephala was fed to cattle ad libitum. However, Sobale et al. (1978) could get digestibility coefficients of 60.1 and 73.3 respectively for dry matter and crude protein with an average daily weight gain of 0.57 kg in growing bulls fed L. leucocephala.

Hulman and Preston (1981) found that Simmental Friesian bulls fed on a basal diet (chopped sugarcane treated with three per cent urea on dry matter basis) supplemented with leucaena at three per cent of live weight, significantly increased the total dry matter intake and growth rate than when supplemented at one per cent or two per cent of body weight.

Teeluck _et al_. (1982) observed faster weight gains in male Zebu cattle when the ration was supplemented with fresh leucaena forage at four per cent of their body weight than at two or five per cent levels.

Wahyuni _et al_. (1982) reported higher body weight gains and low feed conversion ratios in Ongole cattle fed diet containing 40 and 60 per cent leucaena than those fed leucaena alone or 20 per cent leucaena diet. Daily dry matter intakes were significantly higher for the 20, 40 and 60 per cent leucaena diets than for the 100 per cent leucaena or 100 per cent grass diet. Dry matter and protein digestibilities were significantly higher for the 100 per cent leucaena diet than for the others.

According to Dharmaraju (1983) subabul leaf meal can be used successfully to replace groundnut cake upto 40 per cent in conventional rations for lactating Murrah buffaloes.

Rangnekar _et al_. (1983) fed crossbred calves with leucaena forage and Desmonthus forage with limited concentrate mixture and observed daily growth rates of 735 and 543 g respectively. The digestibility coefficients for dry matter, organic matter, crude protein and energy were higher for leucaena diets.

Kurar et al. (1984) could not find any significant difference in the digestibility coefficients of various nutrients in Karan Swiss calves when subabul was fed to replace 25 and 50 per cent respectively of DCP content in the control ration. The efficiency of dry matter utilization was significantly higher in the group fed subabul at 25 per cent level than in the control and 50 per cent groups.

Virk et al. (1985) observed that weight gain, crude protein digestibility and rumen ammonia concentration were significantly lower in buffalo calves when the concentrate part of the ration was replaced by leucaena. Gupta et al. (1986) recorded a significantly higher digestibility of ether extract and crude fibre in the two experimental groups of buffalo calves fed 3.5 and 5 kg respectively of leucaena hay along with 3.5 and 2 kg respectively of wheat straw compared to the control group fed 3.5 kg each of concentrate and wheat straw eventhough there were no significant differences in respect of dry matter, crude protein and nitrogen free extract digestibilities. They recorded a daily gain of 316.2 g and 362.0 g respectively for leucaena fed groups as against 559.4 g for the control group.

Lawar and Patel (1986) concluded from their studies that subabul fodder was efficient to provide 75-100 per cent of crude protein requirements of calves.

Bhaskar _et al_. (1987) reported 22.77 per cent DCP and 48.56 per cent TDN for Hawaiian Giant K-8 when fed to bullocks along with paddy straw. According to them the subabul leaf meal was well accepted by bullocks which recorded a dry matter intake ranging from 2.01 to 2.14 kg with a mean of 2.07 $\pm$ 0.02 kg/100 kg body weight. Further the average digestibility coefficients of dry matter, crude fibre, ether extract and nitrogen free extract of subabaul leaf meal when fed to crossbred calves along with paddy straw worked out to be 49.11, 74.78, 45.33 and 49.16 per cent respectively with calcium and phosphorus balances of 0.55 and 1.15 g/day respectively. Sudhakar (1987) studied the effect of incorporation of _L_. _leucocephala_ at 15, 30 and 45 per cent of the concentrate part of the ration and recorded average digestibility coefficients of 59.92, 60.19 and 61.62 per cent respectively for crude protein, 48.20, 58.40 and 45.63 per cent respectively for ether extract, 59.42, 57.15 and 55.17 per cent respectively for crude fibre and 60.38, 59.33 and 59.92 per cent respectively for nitrogen free extract.

Gopalakrishna (1988) observed significant decrease in digestibilities of crude protein and ether extract as the level of subabul leaf meal was increased in the ration of crossbred calves even though the nitrogen retention was not significantly different.

Gupta et al. (1988) reported DCP contents of 9.66 and 10 per cent and TDN contents of 64.3 and 67.4 per cent respectively from their studies on buffalo calves fed rations containing 20 per cent lucerne and subabul. The average daily gains were 667 and 638 grams respectively for the 2 groups. The digestibility coefficient of all the nutrients were similar and calcium and phosphorus retentions were positive and similar in both groups.

In a growth study carried out on buffalo bulls fed subabul at 50 and 80 per cent levels Gupta et al. (1989) observed dry matter digestibility of 51.62 and 54.4 per cent and crude protein digestibility of 50.57 and 60.87 per cent respectively. The average daily weight gain was, however, only 301 g and 363 g respectively compared to 590 g for the control group fed on concentrate and wheat straw, the reason for the lower weight gain in leucaena fed groups being attributed to lack of available energy.

2.1.3. Subabul and Milk production

Impressive yeilds of milk, both per cow and per hectare, have been reported from leucaena/guinea grass pastures in the tropics (Henke et al., 1950 and Plucknett, 1970). However, in both these studies, the dairy cattle were fed appreciable quantities of concentrates amounting to approximately one half of the total dry matter intakes. Even so, the leucaena/guinea grass pastures at the Mahalona hospital dairy on Kauai, Hawaii were able to carry a stocking rate of 6.1 large Friesian cows per hectare over a period of about 12 years with an annual average milk production per hectare of 9770 kg with no indication of toxicity and no problems with breeding (Plucknett, 1970). Even more impressive was the performance of unsupplemented Jersey cows grazing leucaena/green panic (Panicum maximum var. trichoglume) pastures in southeast Queensland. At a stocking rate of 4.78 cows/ha over a nine-month period, a yield of 6290 kg milk/ha was produced containing 272 kg butterfat and 215 kg protein (Stobbs, 1972).

Improvements in milk production when dairy cows were given small supplements of leucaena in addition to the well-fertilized rhodes grass pasture have been reported by Flores et al. (1979). The increase due to leucaena occurred inspite of the fact that the fertilized pastures of rhodes grass contained over 20 per cent crude protein. The effect of feeding 2 kg leucaena leaf per cow per day was equivalent to feeding 250 g per cow per day of protected protein.

Leucaena is known to taint the milk produced from cows grazing it (Henke, 1958). Such taint can be reduced by ensuring that cows do not graze the leucaena pasture for several hours before milking. Rejection of such tainted milk is not necessary under commercial conditions since the processing of the milk at the factory is sufficient to remove the taint and odour to acceptable levels (Hamilton et al., 1969).

2.1.4. Subabul and beef production

Leucaena cut from natural stands growing in the Philippines and in Indonesia has been traditionally used for fattening cattle. In the Philippines chopped leucaena shoots mixed with rice bran are used for finishing cattle for slaughter. During this phase of fattening, the animals are force-fed using the mixture of rice bran and leucaena as a slurry in water. Similarly, in parts of Indonesia and Timor, cattle are fattened for approximately six months on a diet essentially of leucaena and the pseudostems of bananas. There is complete lack of data on the merit of these feeding systems compared with others, but it is clear from such practices, which have continued for many years, that the local people appreciate the value of leucaena in finishing cattle for slaughter.

In Thailand, the use of leucaena in backyard plantings for supplementing the diets of animals when these are corralled underneath the houses at night is being encouraged (Shelton, 1977). The higher soil fertility around homesteads would enable vigrous growth of leucaena to take place while its value as a supplement during the dry season, in combination with rice straw and crop residues, would be an important adjunct to the diet of both working animals and fattening animals under such conditions.

Experimental results confirm the value of leucaena as a supplement to roughages low in protein to improve animal production. Siebert et al. (1976) showed that supplementing a diet of chopped sugar cane with leucaena, so that the overall protein content of the diet was 9 per cent, gave daily liveweight gains of 0.6 kg/head, identical to that obtained on steers fed sugar cane and meat meal with an overall crude protein content of 10 per cent in the diet.

Steers given fresh leucaena daily as a 20 per cent supplement to sorghum hay also had higher liveweight gains even when the total feed was restricted to 2.5 per cent of the liveweight in all rations (Jones, 1979). After 112 days on the rations, liveweight gain (LWG) of the control was 35 kg, for the animals on 20 per cent leucaena it was 58 kg and for those on 80 per cent leucaena it was 11 kg. Mean daily dry matter intakes for the three diets were 3.85 kg for the control group, 3.91 kg for the animals on 20 per cent leucaena and 1.99 kg for those on 80 per cent leucaena. The efficiency of the diet containing 20 per cent leucaena was far higher (7.62 kg feed/kg gain) than that of the sorghum hay diet.

As a supplement to rice straw, leucaena fed to bulls enabled reasonable gains to be achieved. Daily gains of 0.53, 0.38 and 0.36 kg/day resulted from feedig 60 per cent rice straw + 40 per cent concentrates, 60 per cent rice straw + 40 per cent dry leucaena leaf and 10 per cent rice straw + 90 per cent leucaena leaf respectively (Perez, 1976). The bulls gained little weight when leucaena comprised 90 per cent of the ration than when it contributed only 40 per cent. For fattening animals, a reduction in the amount of rice straw was beneficial - a ration of 50 per cent rice straw + 50 per cent concentrates supported daily liveweight gains of 0.54 kg, compared with 0.71 kg when the ration was composed of 35 per cent rice straw + 35 per cent leucaena leaf + 30 per cent concentrates (Perez, 1976).

Sun-dried leucaena leaf has been used to supplement grazing during the dry season in Malawi. Supplemented steers gained more than the controls but gains were not significantly different from those obtained with a supplement of groundnut cake fed to provide the same level of crude protein as in the leucaena based diet (Thomas and Addy, 1977).

Leucaena has also been used in the intensive fattening rations in combination with forage and energy supplements. In Malawi, zebu and zebu-cross Friesian steers fed a ration of maize stover, maize bran and leucaena to four parts of maize stover and maize bran, gained 1.17 kg per head per day. Used as a protein supplement in this way, leucaena has been as efficient as groundnut cake (Thomas and Addy, 1977). In these rations, leucaena was fed as leaf meal produced by sun-drying the long shoots cut from leucaena. These shoots were dried on the leucaena "stubble", which may be up to 30 cm in height.

Intensive use of molasses and urea for fattening cattle required some form of true protein, preferably by-pass protein, for good liveweight gains (Preston and Willis, 1970). Leucaena, which has fairly low protein solubility in the rumen, proved to be an effective supplement for use with urea-molasses for intensive beef production (Alverez *et al.*, 1977). In addition, the ß-carotene content of leucaena would be of distinct advantage for such high levels of vitamin A for satisfactory animal performance (Preston and Willis, 1970).

In summary, where leucaena has been fed as a protein supplement with other feeds there have been no toxic effects on the animals and the beneficial effects recorded have been comparable with those derived from concentrated protein sources such as groundnut cake and meat meal.

2.1.5. Subabul as a pasture crop

Where leucaena is intended for grazing rather than for cutting, it is usual that the leucaena be grown with an appropriate grass to provide a balanced ration. Grasses that have been found suitable for this purpose are guinea, pangola and signal. Leucaena is usually planted in rows 2-3 m apart with the grass forming an "understorey" between the leucaena rows. Grown in this way, leucaena can be used as a grazed supplement or as the sole pasture for beef or for milk production. As a supplement, the leucaena area would normally be subdivided into four or more areas that can be used in rotation with another sown pasture (Shaw, 1968) or native pasture (Partridge and Ranacou, 1974). In Fiji, paddocks of Nadi blue grass (Dichanthium caricosum) in which zero, 10 or 20 per cent of the area was planted with fertilized leucaena, were grazed at 1.5 steers per hectare to measure animal production. Daily liveweight gains over a three and a half year period were 215 g on the grass pasture, 300 g on the 10 per cent and 500 g on the 20 per cent leucaena pastures (Partridge and Ranacou, 1974).

At Gayndah in Southeast Queensland on clay soils of basaltic origin and in an area with 710 mm annual rainfall, supplemental paddocks of leucaena have resulted in large increases in animal production. On native pastures at a stocking rate of one cow to 3.1 ha, where 0.4 ha of the area was planted with leucaena for winter spring grazing, the cows with access to leucaena had higher weight gains and a conception rate of 80 per cent compared with 59 per cent for controls. Steers allocated at 0.2 ha per head of leucaena in addition to native pasture, gained 114 kg more liveweight over a 17-month period than control steers on native pasture only (Queensland Department of Primary Industries, 1978).

The leucaena area in the supplemented treatments comprised of one paddock to which access was allowed for one to two days per week on the 10 per cent leucaena treatment and three to four days per week for the 20 per cent leucaena treatment. Over the experimental period, gains per head and per hectare declined on the control treatments but remained reasonably stable for the leucaena supplemented treatments. It can be calculated that 1 ha of supplemental browse contributed 600 kg LWG/annum for the 10 per cent leucaena treatment and 800 kg LWG/annum for the 20 per cent leucaena (Jones, 1979).

In another study in subtropical southeast Queensland, remarkably high mean daily gain of 0.93 kg/head/day for two-year-old steers and 0.88 kg/head/day for calves over a 200-day grazing period was recorded on leucaena/ Nandi setaria pastures. These animals received only iodized salt supplements (Jones, 1973). When slaughtered, the steers had very yellow fat, markedly more pigmented than normal pasture-fed steers and undoubtedly due to the high levels of carotene in the leucaena leaves.

The wide range in mean daily gain (0.29 to 0.93 kg) when steers graze leucaena-based pastures is associated with several factors. Environmental factors that influence leucaena growth are important, not only in determining the yield level and yield distribution throughout the year, but also in influencing the mimosine levels in the material grown. The intake of leucaena and the development of toxicity are also important and, perhaps, the most likely causes of the low gains reported on vigorous leucaena-based pastures in hot tropical areas.

2.1.6. Nutritive value of Subabul for sheep and goat

Joshi and Upadhyay (1976) recorded 15.5 per cent DCP and 54 per cent TDN for leucaena in the case of sheep.

Yerana et al. (1978) reported the dry matter digestibility of Leucaena leucocephala in sheep as 59.7 per cent with a dry matter intake of 3.66 per cent of body weight and nitrogen retention of 12.1 g/day.

According to Devendra (1982) digestibility co-efficients of dry matter, crude protein and crude fibre contents of subabul were 50 to 51.5, 40.5 to 46.3 and 31.2 to 60.2 per cent respectively in the case of sheep. He found that the DCP, TDN, digetible energy and metabolizable energy values of subabul ranged from 9.1 to 10.1 per cent, 46.7 to 54.2 per cent, 2.06 to 2.039 Mcal/kg and 1.69 to 2.0 Mcal/kg respectively. He concluded that inclusion of 50 per cent L. leucocephala forage was optimum for sheep. From the studies on the effect of replacement of ground nut cake nitrogen with subabul leaf nitrogen at 0, 33, 67 and 100 per cent levels in complete rations for lambs, Vahidulla (1984) found that the dry matter intake was significantly higher in the case of rations with 100 per cent subabul leaves and average daily gain was 29 per cent more. It was concluded that ground nut cake nitrogen can be completely replaced with subabul nitrogen in the ration for lambs.

Upadhyay et al. (1974) reported DCP and TDN values of 16.73 and 70.22 per cent respectively for L. leucocephala when fed as sole feed to adult Barbari bucks. Digestibility coefficients of 67.5, 70.7, 46.8, 54.4 and 73.2 respectively for dry matter, crude protein, ether extract, crude fibre ad nitrogen free extract were reported by James (1978) for L. leucocephala when fed to Saanen Malabari kids, the DCP, TDN and Nutritive ratio being 12.87, 66.11 and 4.1 respectively. The kids showed an average daily gain of 26.2 g with an average daily dry matter intake of 3.9 kg/100 kg live weight.

According to Devendra (1982) the digestibility co-efficients of dry matter, crude protein and crude fibre contents of subabul were 53.9 to 56.4, 44.8 to 45.0 and 38.5 to 64.8 per cent respectively in the case of goats. He found that the DCP, TDN,digestible energy and metabolizable energy values of subabul ranged from 9.3 to 11.0 per cent, 46.9 to 67.8 per cent, 2.07 to 3.02 Mcal/kg and 1.7 to 2.47 Mcal/kg respectively in goats. He concluded that inclusion of 50 per cent L. leucocephala forage in the diet was optimum for goats.

Johri et al. (1983) studied the effect of feeding subabul at 50, 75 and 100 per cent levels to Barbari bucks in three phases and found that the digestibility coefficients of all the nutrients were higher in the last phase with DCP and TDN values of 18.8 and 57.9 per cent respectively.

Jaikishan et al. (1986) studied the effect of replacement of 25 and 50 per cent respectively of crude protein in the concentrate mixture fed to the control group of goats with L. leucocephala. The digestibility co-efficients for the control, 25 per cent and 50 per cent leucaena groups worked out to be 63.78, 60.28 and 56.24 respectively for dry matter, 57.53, 52.9 and 38.46 respectively for crude protein, 65.82, 45.54 and 33.99 respectively for ether extract, 49.19, 49.56 and 48.50 respectively for crude fibre and 76.33, 72.21 and 71.26 respectively for nitrogen free extract. The retention of nitrogen, calcium and phosphorus in g/day worked out to be 5.61, 1.21 and 0.41 for the control, 4.27, 1.97 and 0.44 for 25 per cent leucaena group and 3.73, 2.13 and 0.75 for 50 per cent leucaena group respectively.

Kumar et al. (1987) fed two groups of five male Beetal X Black Bengal goats with L. leucocephala hay (group 1) and Trifolium alexandrium hay (group 2). The DCP and TDN values were 12.61, 13.04 per cent and 51.52, 52.45 per cent for group 1 and 2 respectively. Daily dry matter intake was 2.92 and 3.52 kg/100 kg body weight and average daily gains were 34.9 and 35.5 g for groups 1 and 2 respectively. Dry matter intake, growth rate, nutrient digestibility, carcass traits and semen characteristics were not significantly different for different diets. They concluded that L. leucocephala could be used as the sole feed for goats.

The DCP and TDN values of 15.77 and 69.57 per cent were reported by Chakraborty and Ghosh (1988) for subabul in Black bengal goats with mean digestibility coefficients of 73.50, 86.72, 75.06, 70.23, 67.00, 53.89, 20.74, 38.80 and 78.68 for dry matter, crude protein, ether extract, crude fibre, nitrogen free extract, neutral detergent fibre, cellulose, acid detergent fibre and hemicellulose respectively. They reported an average dry matter intake of 2.46 per cent of body weight in Black Bengal goats fed fresh L. leucocephala foliage ad libitum for 56 days.

Mtenga and Shoo (1990) conducted an experiment with 6 to 7 months old grazing indigenous small East African goats. They were given only hay (H), hay and leucaena 100 g (HL_1), hay and leucaena 200 g (HL_2) and hay and leucaena ad libitium (HL_3). Daily weight gains were 20, 23, 29 and 30 g for goats under H, HL_1, HL_2 and HL_3 groups respectively. Crude fibre digestibility decreased and crude protein digestibility increased with increased levels of leucaena in the diet.

From the studies carried out in Barbari bucks fed on subabul hay as the sole feed, Yadav et al. (1990) concluded that the digestibility of organic nutrients were satisfactory and that all bucks maintained positive balance of nitrogen, calcium and phosphorus. The DCP and TDN values of subabul worked out to be 14.44 and 54.64 per cent respectively with a nutritive ratio of 1: 2.78.

2.2. Mimosine content in subabul

The use of subabul as an all purpose livestock feed has been limited by the presence of mimosine (ß-[N-(3-hydroxy-4-pyridone)]-α-amino propionic acid) (Hegarty et al., 1964; Hylin, 1964), a toxic, non protein amino acid that causes low weight gains, general poor condition and hair loss in ruminants and non ruminants (Kingsbury, 1964 and Hegarty et al., 1976). Mimosine may act as an amino acid antagonist or as an enzyme inhibitor through complex formation and chelation (Hegarty, 1978). There appear to be 2 distinct adverse effects of leucaena feeding - an acute effect with a rapid deterioration in condition associated with hair loss, excessive salivation and catarrhal conjunctivitis (Hegarty et al., 1964 and Vohradsky, 1972) and a chronic effect in which the clinical signs develop more slowly and may take many months under grazing condition (Jones et al., 1976). A consistent feature associated with the chronic effect has been the development of goitre (Bindon and Lamond, 1966) and a marked depression in the serum thyroxine (T_4) levels (Jones et al., 1976, 1978). The active goitrogen has now been identified as 3-hydroxy-4-(1H)-pyridone (DHP), which is produced readily by the hydrolysis of the toxic amino acid mimosine in the rumen (Hegarty et al., 1979).

While Hamilton et al. (1971) reported that sun dried L. leucocephala leaves contained about 1.2 per cent of mimosine on dry matter basis, Megarrity (1978) recorded 1.3 to 4.1 per cent of mimosine in L. leucocephala on dry matter basis. According to Sobale et al. (1978) leucaena contained, on an average, 1.5 per cent of mimosine on dry matter basis. Studies carried out by Jones (1979) showed that mimosine occurs in all parts of leucaena plant and that the concentration in growing tips may attain a level of 12 per cent, in young leaves 3 to 5 per cent, in pods 3 to 5 per cent and in seeds 4 to 5 per cent on a dry matter basis. Green stem may contain upto 2 per cent of mimosine but older stem that has become suberous has usually less than 1 per cent of mimosine in the dry matter (Jones, 1979). While fresh L. leucocephala contained 2 to 9 per cent mimosine, its concentration was 7.2 to 40 per cent less after drying (Jones, 1980). Wood et al. (1983) recorded 3.2 per cent mimosine in sun dried L. leucocephala meal. Hongo et al. (1983) concluded from their studies that on an average L.

leucocephala had 3 to 6 per cent of mimosine in the whole plant and that the value was higher for the segment of active growth.

Rangnekar et al. (1983) found that subabul at 10, 20, 30 and 40 days of regrowth contained 4.6, 4.2, 3.2 and 2 per cent respectively of mimosine on dry matter basis.

Akbar and Gupta (1984) analysed 9 cultivars of L. leucocephala and found that the mimosine content varied from 2.68 (K-8) to 3.19 (Australian common) per cent when the samples were dried at 40°C, while it ranged from 2.37 (K-8) to 2.86 (Australian common) per cent at 100°C. Three varieties (L. glauca, Hawaiian giant and Australian common) contained more than 3 per cent mimosine, while the two (K-8 and K-28) had lowest percentage of mimosine . The mimosine content is reduced by 9.45 to 14.52 per cent, when the temperature for drying the samples is raised from 40°C to 100°C. Reduction in mimosine content by heat treatment has also been reported in earlier studies (NAS, 1977 and Jones, 1981). James and Gangadevi (1990) found that, ensiling is an effective method to reduce mimosine content in L. leucocephala leaves without altering the bioavailability of nutrients.

The mimosine content of different parts of L. leucocephala (Cv. K-8) ranged from 1.38 per cent in dry pods to 5.06 per cent in immature leaves with intermediate values of 2.96, 2.21, 1.83, 2.37, 5.04, 4.94, 3.60 per cent respectively in mature leaves, branches, stems, green pods, seeds, buds and flowers on a dry matter basis (Akbar and Gupta, 1984). While according to Dogra et al. (1986) the concentration of mimosine in leucaena ranged from 0.86 to 1.73 per cent on dry matter basis, values of 3.02, 2.2, 1.8, 1.7, 2.5 and 4.04 per cent respectively by Kewalramani et al. (1987), Sudhakar and Ramarao, (1987), Balagopal and Ravi, (1988), Gupta et al. (1988), Gupta et al. (1989), Sunaria and Vidyasagar, (1989).

2.3. Toxicity of leucaena for ruminants

The obvious clinical signs of toxicity in the ruminant is alopecia (hair loss), which occurs sporadically when cattle are introduced to leucaena for the first time or when they receive rations containing high amounts of leucaena. In addition to the alopecia noted when animals first have access to leucaena, there are other reports in the literature of toxicity in animals that have grazed leucaena for long periods. Clinical signs include alopecia, loss of appetite, excessive salivation, incoordination of gait, enlarged thyroid glands, poor breeding performance and the production of goitrous calves that die at birth (Compere, 1959; Letts, 1963; Vohradsky, 1972; Jones et al., 1976; Holmes, 1976 and Jones et al., 1978).

Sheep unaccustomed to leucaena, shed their wool approximately 7 to 14 days after the commencement of leucaena feeding and continued heavy feeding may result in death (Hegarty, et al., 1964; Reis et al., 1975). Large thyroids were also noted in lambs born to ewes fed leucaena (Bindon and Lamond, 1966), but Little and Hamilton, (1971) were unable to repeat this observation. Even though goats are said to be unaffected (Kraneveld and Djaenoedin, 1947; Owen, 1958), experiments with goats in Queensland have shown that the goats are affected by leucaena and shows hair loss similar to that encountered with cattle (Jones, 1979). In a study (Shiroma and Akashi, 1976) rumen fluid from goats degraded the mimosine in leucaena tips from 60 to 0.3 mg/g after 25 hours and, with pure mimosine, 98 per cent was degraded by the rumen fluid after 5 hours. If ruminants are gradulally introduced to leucaena, rumen microorganisms capable of breaking down the mimosine increase so that the toxic action of mimosine no longer poses a problem (Jones, 1979).

High levels of selenium were once thought to be implicated in the toxicity symptoms exhibited by experimental animals fed on leucaena (Arnold, 1944; Gardner and Bennetts, 1956). This hypothesis has not been substantiated either in Hawaii (Yoshida, 1945) or in Australia (Hutton and Gray, 1959). The leaves are reported to contain hydrocyanic acid (HCN) (Oakes, 1968) but its presence in leucaena has not been substantiated in Australia, where it had been considered as a possible reason for the enlarged thyroid glands noted in animals grazing leucaena pastures (Jones, 1979).

It is now apparent that the chronic toxicity of leucaena for ruminants is related only indirectly to its mimosine content. Generally speaking, mimosine toxicity per se is not the major problem. It has now been shown that DHP (3-hydroxy-4-1(H)-pyridone), the breakdown product of mimosine in the rumen, is a potent goitrogen (Hegarty et al., 1976). DHP may also have other undesirable effects but its goitrogenicity is now without question. Circulating DHP prevents iodination of tyrosine, the first step in the synthesis of thyroxine, resulting in goitre and reduced levels of thyroxine (T_4) in the serum. The depressed thyroxine level has other side effects that may be associated with the reduced appetite, drooling and even hair loss that were noted in cattle fed high levels of leucaena in Australia and New Guinea (Jones et al., 1976).

All clinical signs are not consistently present when animals are fed diets high in leucaena. For example, Hamilton et al. (1971) reported that leucaena did not affect the length of oestrous cycle, conception rate or gestation length in heifers. The only adverse effects noted were slight depilation, some mild incoordination and nervous symptoms occurring briefly during gestation, and lower mean birth weight of calves (19.6 kg versus 25.8 kg for the lucerne control diet). In calves thyroid glands were also enlarged and plasma-bound iodine levels elevated. The histology of the thyroid gland was, however, normal.

The lack of any adverse effect on conception rates in the study of Hamilton et al. (1971) confirmed the work of Henke et al. (1950) in Hawaii where, for three successive lactations in cows fed diets of leucaena and concentrates, the animals bred normally. They are also in accordance with the results from an experiment extending over five years in which Hereford and Hereford x Afrikander heifers that were reared and mated, on leucaena -based pastures gave conception rates of over 90 per cent (Jones, 1979). However, Holmes in New Guinea reported low conception rates and poor growth in Brahman cross heifers grazing a predominantly leucaena pasture containing some unpalatable weeds (Holmes, 1976). These apparently conflicting reports may be the result of the different intakes of mimosine by the cattle in the different experiments. For example, in the study by Hamilton et al. (1971) the overall mean concentration of mimosine in the dry material ranged from 0.34 to 1.2 per cent, while mimosine values of 3 to 4 per cent were reported in studies where problems have occurred (Jones et al., 1976; Jones et al., 1978). The conflicting results for heifers in southeast Queensland and in New Guinea could also be due to the fact that in the Australian study the animals grazed a fertilized mixed pasture of leucaena and setaria whereas those in New Guinea were confined to unfertilized leucaena.

Unfortunately the mimosine concentration of the diets fed has not been measured in many of the earlier experiments. Part of the variability encountered between animals grazing the same leucaena pasture is undoubtedly due to the different proportion of leucaena selected by the different animals. Estimation of the proportion of leucaena selected by 10 steers grazing the same leucaena/pangola pasture, using C^{12}/C^{13} ratio in the faeces (Jones et al., 1979) showed that individual animals selected from 30 to 70 per cent of leucaena in the diet. Furthermore, animals that selected lower amounts of leucaena had low toxicity scores and those that selected high amounts of leucaena had high toxicity scores (Jones, 1979).

In studies where the level of leucaena in the diet of steers varied from zero to 100 per cent, rations containing up to 40 per cent of leucaena resulted in good liveweight gain over a period of 112 days although the T_4 levels in the serum of cattle on the 40 per cent leucaena diet decreased to less than 20 n mol/litre by the end of the feeding period. Steers on the zero per cent, 10 per cent and 20 per cent leucaena diets maintained normal T_4 levels of 60-100 n mol/litre throughout (Jones, 1977). It is, therefore, suggested that diets containing less than 30 per cent of leucaena would not be expected to cause toxicity problems in growing stock.

One notable feature of the toxicity of leucaena to animals is its ready reversibility when the animals are removed to a non-leucaena pasture. Cessation of leucaena feeding results in a rapid return to normal T_4 levels and, provided the animals have not been unduly stressed prior to the change of feed, their growth rates return to normal (Jones et al., 1978). The toxicity is not acute under field conditions - there is no known record of animals dying while grazing leucaena - deaths that have occurred were reported from pen feeding studies where leucaena was fed as the sole ration. Under field conditions, sick animals are recognized readily and can be removed to non-leucaena pastures. In this respect, white clover and lucerne, which cause bloat in temperate pastures, are a far greater risk in terms of stock losses than is leucaena.

2.4. Degradation of mimosine

Tahahashi and Ripperton (1949) postulated for the first time that mimosine was destroyed in the rumen. However, Damseaux (1956) maintained that this hypothesis was incorrect because he found that sheep could not readiy eat the plant and the small amount which was eaten by them caused shedding of fleece in 10 to 14 days eventhough they could not produce direct evidence showing whether or not mimosine was degraded in the rumen. Although Hegarty et al. (1964) reported that mimosine administered into the rumen of sheep was detected as 3,4-DHP in the urine, no information dealing with the direct effect of rumen micro organisms on the degradation of mimosine was available. Shiroma and Akashi (1976) observed that goat rumen fluid is able to degrade mimosine in L. leucocephala (Lam.) de. Wit.

Tangendjaja et al. (1983) fractionated rumen fluid from sheep fed on different diets into micro organisms and supernatent fractions and the formar divided into bacteria - rich and protozoa - rich fractions. They found that rumen fluid from sheep fed on a lucerne - oats mixture produced a more rapid degradation of mimosine than did that from sheep fed on lucerne hay, which was greater than that from a Digitaria pentzii diet. Most activity was in the bacteria - rich fraction for the lucerne - oats diet and in the protozoa - rich fraction for the other diets. The rate of degradation of endogenous mimosine in leucaena leaf during incubation in rumen fluid was much greater than that for the purified mimosine. The substantial degradation observed when a buffer solution was substituted for rumen fluid was attributed to endogenous leaf enzymes. These enzyme systems were more efficient in degrading mimosine than the micro organisms in the rumen liquor.

Mimosine is degraded quite rapidly to 3,4 - DHP by an enzyme present in macerated green leaves (Lowry et al., 1983). Virtually more than 80 per cent of mimosine is degraded by heating of intact leucaena leaves at 70°C for 10 minutes (Tangendjaja et al., 1984). DHP though less toxic than mimosine is goitrogenic. (Hegarty et al., 1976).

From their studies on crossbred bulls in India fed leucaena at 0,50 and 100 per cent levels, Badve et al. (1985) found that mimosine excretion and serum protein bound iodine levels were in agreement with those reported by Jones and Megarrity, (1981) for goats fed leucaena in Hawaii indicating breakdown of mimosine and DHP to nontoxic compounds thereby preventing toxicity as suggested by Jones and Lowry, (1984).

Jones and Megarrity (1983) fed goats both in Australia and Hawaii, comparable diets of leucaena and lucerne (control) over a 7 weeks period. Intake of mimosine, excretion of ruminal metabolite 3,4 DHP and serum concentrations of thyroxine (T_4) were measured in both studies. Mean mimosine intakes on the highest leucaena diets in Australia and Hawaii were similar at about 20g day^{-1}. In Australia goats on the all leucaena diets became hypothyroid after only 3 weeks of feeding, thyroid glands were enlarged and erosions of oesophageal mucosa and reticulorumen occurred. Excretion of DHP in the urine was related to the daily mimosine intakes with recoveries of about 86 per cent. In marked contrast, goats fed on leucaena in Hawaii exhibited no clinical signs of toxicity and excreted less than 1 per cent of the mimosine intake as DHP in the urine. No degradation of DHP occurred *in vitro* with rumen fluid from Australian goats, where as 71 per cent of the added DHP was degraded after 5 hr of incubation with rumen fluid from goats in Hawaii. The results support the hypothesis that the differences observed are attributed to a different microbial metabolism of mimosine and DHP in ruminants in Hawaii. It is of interest that feed intake was increased immmediately when ruminal degradation of DHP was induced in leucaena fed goats (Jones and Lowry, 1984).

Microbial detoxification of mimosine was detected during *in vitro* studies with rumen fluid from cattle and sheep in Canada (Kudo et al., 1984). Mimosine was degraded rapidly (2.17 $\mu g.mL^{-1}.h^{-1}$) by rumen inocula from sheep on a concentrate diet but inocula from sheep on cubed hay showed much less activity (0.44 $\mu g.mL^{-1}.h^{-1}$). Mimosine was also metabolized by rumen microorganisms from steers on concentrate rations (2.88 $\mu g.mL^{-1}.h^{-1}$). But rates were lower when inocula originated from cattle fed hay pasture or silage diet (<1.87 $\mu g.mL^{-1}.h^{-1}$). They concluded that in Canada rumen fluid contains microorganisms capable of mimosine degradation and that rate of detoxification are enhanced by rumen microbiota from sheep and cattle on concentrate diets. Even though the major metabolite of mimosine in ruminants fed leucaena is 3,4 - DHP, occasional appearance of another compound detectable by the formation of a blue complex with ferric ions in the urine was reported by Jones and Megarrity (1983) and the same was later on determined to be 3-hydroxy-2(1H)- pyridone (2,3-DHP), a structural isomer of 3,4-DHP (Ford et al., 1984).

Mixed cultures of rumen bacteria from adapted leucaena fed goats or leucaena fed cattle are capable of completely degrading both DHP isomers, and one particular culture has also been found which converts 3,4-DHP to 2,3-DHP (Jones et al., 1984). The sporadic appearance of significant quantities (>0.1 per cent) of 2,3-DHP in the urine of leucaena fed ruminants may, therefore, be attributed to fluctuations in the relative population of bacteria responsible for the sequential steps of mimosine degradation.

Quite contrary to the previous report Raurella and Jones, (1985) found that leucaena toxicity was no longer a problem for cattle at Erap, because they then had the specific rumen microorganisms acquired to degrade DHP, probably due to some mutation in the bacteria in the cattle of Australian origin or because such bacteria were acquired from other cattle. According to Jones (1984) DHP degrading organisms can be introduced in animals by drenching them with fresh rumen fluid taken from those known to degrade DHP. Jones et al. (1985) showed that the transfer of DHP degrading ability is possible by rumen infusion with bacterial cultures; that animal to animal transfer occurs in small paddocks; that the introduced bacteria are being maintained when leucaena is fed and that they persist in the animal for at least 6 months after removal from leucaena feeding.

Sriskandarajah and Komolong (1986) recorded complete absence of 3,4-DHP, although its structural isomer 2,3-DHP, was found at 0.07 and 0.6 per cent in the urine of sheep and goat respectively fed leucaena hay in Papua New Guinea indicating that microbes converting 3,4-DHP to 2,3-DHP exist in the rumen of these animals. Low levels of DHP in the urine of sheep and goat ingesting high levels of leucaena in Papua New Guinea indicate their ability to degrade mimosine and DHP through the presence of appropriate rumen micro organisms. Brahman cattle at the Portorial Research Centre, Erap, Papua New Guinea which was allowed to mix with a herd of Javanese Zebu cattle introduced to the centre, were found not to excrete DHP in their urine. These findings illustrate the existence of DHP degrading micro organisms in the rumen of sheep, goat and cattle introduced from Asia into Papua New Guinea.

Sunil Kumar et al. (1987) studied the *in vitro* degradation of chemically pure mimosine by rumen fluid and its fractions obtained from buffaloes maintained on wheat straw, green forage, crushed maize grains and mustard cake. The whole rumen fluid degraded mimosine to the extent of 100 per cent when incubated for 50 hours. Amongst the rumen fluid fractions, bacteria were more active than protozoa. Cell free supernantent was ineffective. At 50 hour of incubation bacteria and protozoa degraded mimosine to the extent of 87 and 28 per cent respectively. Mimosine at 2.12 mg ml^{-1} rumen fluid concentration did not affect rumen microbial activity. Ammonia and volatile fatty acids were formed from the degradation of mimosine.

Dominguez-Bello (1989) reported increased ammonia concentration in the rumen of sheep when leucaena was added to the diet. According to Neeru et al. (1992) the higher concentrations of ammonia in the rumen fluid after feeding leucaena indicates the presence of more soluble proteins and/or free amino acids which are easily degraded in the rumen to produce ammonia. Greater availability of ammonia due to the degradation of mimosine may be one of the reasons for higher microbial growth after feeding leucaena. Neeru et al. (1992) examined rumen microbial activity for finding out the toxic effects after feeding L. leucocephala (K-8) at 20 per cent of the diet of buffalo calves and found that there was no adverse effect on rumen microbial activity, while levels of TCA-N, total N and NH_3-N increased, indicating an overall positive effect. There was, however, a decrease in the concentration of total volatile fatty acids in the rumen fluid.

Materials and Methods

MATERIALS AND METHODS

3.1. Location

The study "Microbial degradation of mimosine in goats" was carried out in the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy. The experimental goats were maintained in the metabolism stall of the Departmentt of Animal Nutrition, under standard conditions of feeding and management.

3.2. Experimental programme

Samples of edible parts of subabul viz., immature leaves, mature leaves, tender stems and seeds were analysed for their proximate chemical composition and mimosine content during the months of May, June and July. *In vitro* degradation of chemically pure mimosine and that of different edible parts of subabul was determined by artificial rumen technique using three rumen fistulated goats.

3.2.1. Experimental animals

Three Saanen x Malabari adult healthy goats weighing about 20 kg were used for fixing the rumen fistula for the collection of rumen fluid to be used for the determination of *in vitro* degradation of mimosine.

3.2.1.1. Surgical technique

The animal was fasted for 24 hours prior to the operation. It was controlled on right lateral recumbency and the upper left flank region was prepared for operation. Local infiltration anaesthesia was induced by injecting about 4 - 6 ml of 2 per cent lignocaine hydrochloride solution subcutaneously.

A circular area over the skin about 3 cm in diameter, was marked midway between last rib and tuber coaxe, ventral to the lumbar vertebrae on the left side. A cruciate incision was made on the skin and the four flaps were dissected out, to make a circular incision. The fascia, muscles and the peritoneum were incised to enter the abdominal cavity. The peritoneum was stretched and fixed to the skin by a series of mattress sutures. By gentle traction, the rumen wall was brought in level with the abdominal incision and anchored by means of four stay sutures. The exposed portion of the rumen was then sutured to the abdominal incision, by inserting a series of mattress sutures without piercing the mucous membrane of the rumen, using sterile silk. The stay sutures were then removed and the area was covered with sterile gauze and was daily dressed with nitrofurazolidone ointment. On the 7th day, the sutures were removed. The rumen wall was excised 0.5 cm away from the sutured margin, all around.

The cylindrical portion of the canula fitted with one of the threaded circular discs with the rubber washer was pushed into the rumen. The other circular disc with its rubber washer was then fixed on the protruding portion of the canula. The opening portion of the canula was closed with a screw cap. Nitrofurazolidone ointment was applied over the wound daily. Streptopenicillin 4 lakhs IU was administered intramuscularly for 5 days post operatively. The wound healed up within a week. The adhesion between the rumen and abdominal wall was good.

3.2.2. Experimental diet

Ration for experimental goats was computed as per feeding standards (ICAR, 1985).

Table 1. Ingredient composition of the concentrate fed to goats.

Ingredients	Per cent
Ground nut cake	20.0
Maize	27.0
Wheat bran	30.0
Horse gram	20.0
Mineral mixture	1.5
Salt	1.5

Table 2. Proximate chemical composition of the concentrate fed to goats on dry matter basis.

Proximate principle	Per cent
Dry matter	91.6
Ether extract	5.8
Crude protein	16.8
Crude fibre	11.6
Total ash	8.8
NFE	57.0

3.2.3. Management

The experimental animals were housed individually with facilities for feeding and watering. Weighed quantities of concentrate and roughage were offered daily. The concentrate was given at the rate of 300 g/head/day. Roughage (Hybrid Napier) was given at the rate of 3 kg/head/day. Wholesome water was provided *ad libitum* .

3.2.4. Determination of mimosine in leucaena samples

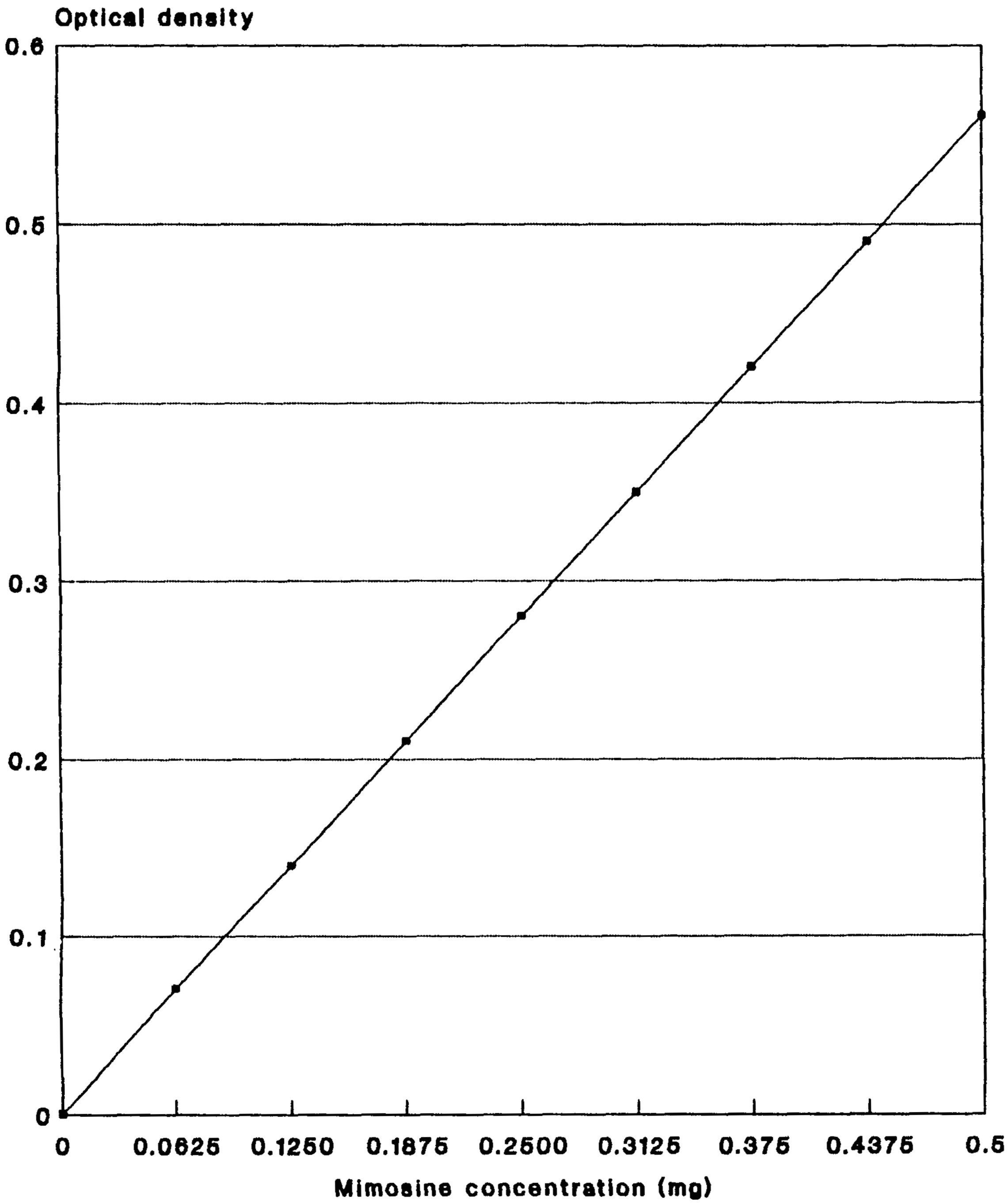
Mimosine content of immature leaves, mature leaves, tender stems and seeds of subabul was estimated using the method described by Brewbaker *et al*. (1981).

One g of air dried ground leucaena sample was weighed out in to a 100 ml volumetric flask, and made up the volume with 0.1 N HCl and then macerated in a homogenizer. Ten ml aliquote of the macerate and 15 ml of 0.1 N HCl containing charcoal was taken in a test tube and heated in a boiling water bath for 15 minutes. It was then filtered through Whatman No. 2. filter paper. To 2 ml of the above filtrate, 5 ml of EDTA solution (1 g Na_2 EDTA . 2 H_2O in 4L of distilled water) and 1 ml of ferric chloride reagent (4 g $FeCl_3$. 6 H_2Oin 500 ml of 0.1 N HCl) were added. The blank and standards containing 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75 and 2 ml of pure mimosine (0.25 mg/ml) were prepared similarly. They were kept in dark for 15 minutes. The absorbance was determined at 535 nm in a Spectronic 20 - spectrophotometer. The mimosine contents of the samples were found out from the standard curve (Fig. 1).

3.3. Collection of rumen liquor

Samples of rumen liquor were taken from the fistulated goats before offering the concentrate mixture in the morning and 2 hours after they had consumed the concentrate mixture. The collection of rumen liquor was done in a 250 ml conical flask by inserting a hard polythene tube into the rumen after opening the cap of the fistula and sucking out the ruminal fluid from different depths and directions. The conical flask was closed with a double bore rubber cork

Fig. 1. Calibration curve for mimosine content in leucaena samples



containing rumen liquor without mimosine were set up. Likewise, instead of mimosine solution, samples of ground immature subabul leaves (75 mg) containing 9.95 per cent mimosine, mature leaves (152 mg) containing 4.91 per cent mimosine, tender stems (225 mg) containing 3.36 per cent mimosine and seeds (79 mg) containing 9.51 per cent mimosine were taken for the study of mimosine degradation.

Mimosine concentrations at 12 hr intervals were determined colorimetrically. Each subsample (0.5 ml) was mixed with 0.05 ml ferric chloride reagent (5.4 g $FeCl_3$. 6 H_2O in 100 ml of 2.5 N HCl) and 4.45 ml water. This mixture was centrifuged at 6000 rpm for 5 minutes and the absorbance of supernatent clear solution was measured at 535 nm. The mimosine content was determined from the standard curve. The standards containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 ml of mimosine (0.375 mg/ml) were used for the preparation of standard curve (Fig. 2).

3.4. Chemical analysis

3.4.1. Proximate composition

The proximate principles of conentrate, roughage and samples of leucaena were determined as per AOAC (1980).

fitted with glass tubes. One of the glass tubes was connected to the free end of the polythene tube and the other to a rubber tubing to suck out the rumen liquor. About 80 ml of rumen liqour was collected from each goat at each sampling time and were mixed thoroughly. It was immediately brought to the laboratory for further processing and analysis.

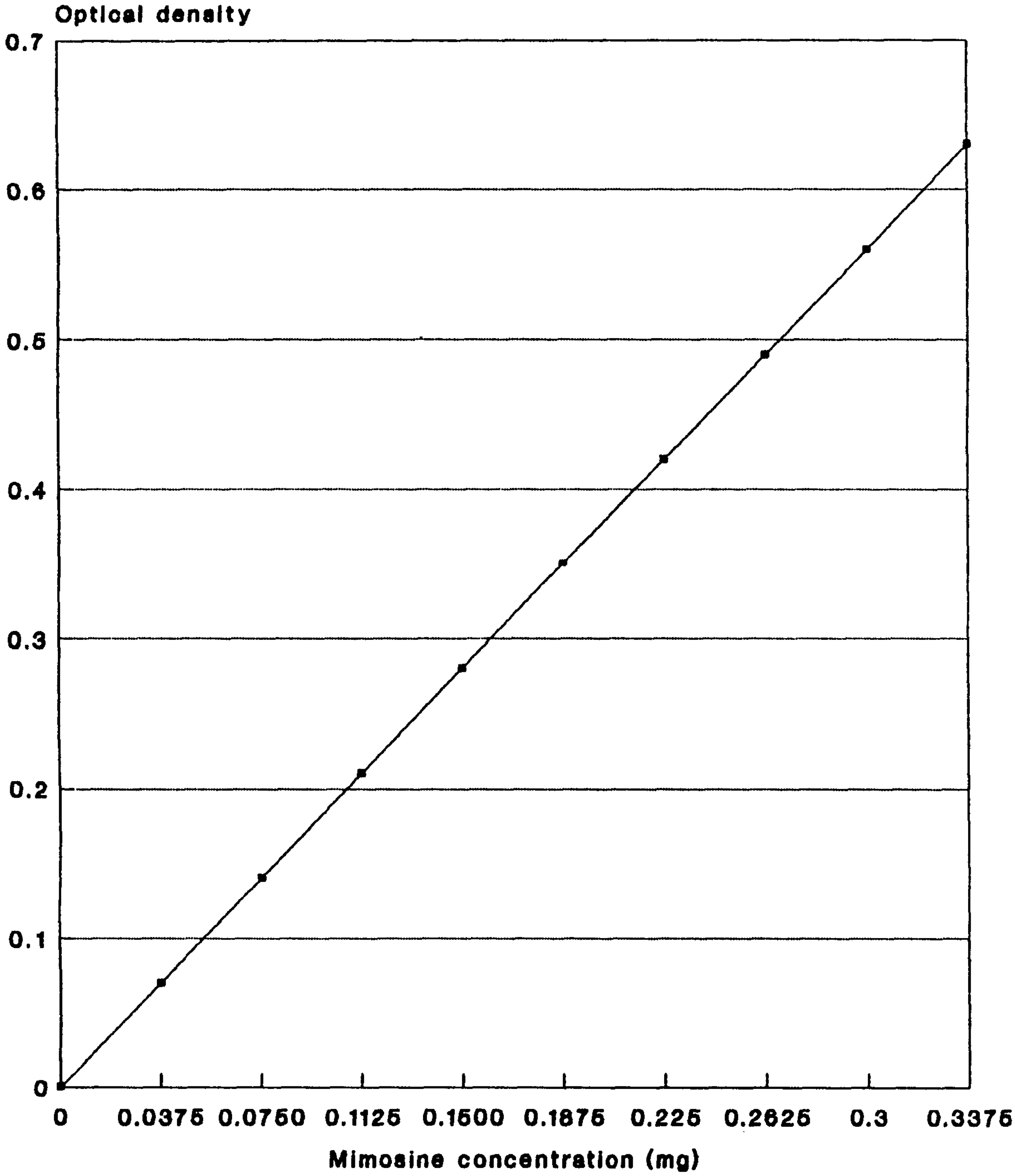
3.3.1 Artificial rumen technique

A 250 ml conical flask was fitted with a double bored rubber cork. Through the holes glass tubes were inserted and one was used as inlet for carbondioxide and the other served as the outlet. Rubber tubes were fixed to the glass tubes and a Bunsen valve was made in the outlet of the glass tube. Clips were used for opening and closing the tubes.

3.3.1.1. Degradation of mimosine in rumen liquor

The rumen liquor was strained through four layers of muslin cloth. From this, a 17 ml aliquote and 3 ml of mimosine solution (2.5 mg pure mimosine per ml) were added to the conical flask and the same was flushed with CO_2. The samples were incubated at 39°C under anaerobic conditions and subsamples (0.5 ml) were assayed at 0, 12, 24, 36, 48 hr to determine the mimosine concentration. Controls

Fig. 2. Calibration curve for mimosine degradation in SRL of goat



3.4.2. VFA estimation

The total VFA content of rumen liquor incubated with samples was estimated at 12 hr intervals using the method described by Barnett and Reid (1956) with minor modifications.

After collection, the rumen liquor was strained. From this, a 34 ml aliquote was taken in a 250 ml conical flask and the subabul sample containing 15 mg of mimosine was added and incubated at 39°C. At 12 hour intervals, 5 ml was taken from each flask and was centrifuged for 5 minutes. From the clear solution, 2 ml was taken and 0.1 ml of $HgCl_2$ was added to arrest microbial activity. To this 1 ml of liquid paraffin containing 2 per cent n-octyl alcohol was added and then introduced into the Kjeldhal steam distillation apparatus. Two ml of Scaris Brick buffer (1 ml of 10% Potassium oxalate + 1 ml of 5% oxalic acid) was also introduced and distillation was carried out till 150 ml of distillate was obtained. It was titrated against 0.01 N NaOH in the presence of phenolphthalein indicator. A blank using 2 ml distilled water was also determined simultaneously. TVFA was calculated as millimoles of acetic acid.

3.4.3. Ammonia estimation

Ammonia content in the rumen liquor containing samples of leucaena was estimated using conway diffusion technique as described by Conway (1957).

From the strained rumen liquor, a 34 ml aliquote was taken in a 250 ml conical flask and the leucaena samples containig 15 mg mimosine was added and incubated at 39°C. From this, at 12 hr intervals, 1 ml portion was withdrawn and placed in one side of the outer chamber of the Conway diffusion dish and 1 ml of potassium carbonate (45 %) was placed in the opposite side of the outer chamber. One ml of boric acid reagent [prepared by mixing 100 ml of 10 % boric acid, 1 ml 0.1 % methyl red (in ethanol) and 8 ml of 0.1 % bromocresal green (in ethanol) and diluting to 1 litre with distilled water] was placed in the inner chamber of the dish. Then the dish was closed with the cover and was gently rotated on a flat surface to ensure mixing of potassium carbonate (45 %) with the contents. Then it was allowed to diffuse for 20 minutes. After diffusion, the ammonia absorbed by the boric acid was titrated against 0.001 N H_2So_4 . A blank was also run simultaneously.

3.5. Statistical analysis

Statistical analysis of the data was carried out as per methods described by Snedecor and Cochran (1967).

Results

RESULTS

The results obtained during the course of investigation are detailed under following heads.

4.1. Proximate chemical composition of leucaena.

Data on proximate chemical composition of different edible parts of *L. leucocephala* viz.immature leaves,mature leaves,tender stems and seeds are given in Tables 3 to 6, summarised data in Table 7.

4.2. Mimosine content of leucaena.

The mimosine concentrations of different edible parts of leucaena for the months of May,June and July are presented in Table 8 to 10 and Fig.3., summarised data in Table 11 and statistical analysis in Tables 12 and 13.

4.3. Mimosine degradation.

The data on mimosine concentrations at different time intervals of incubation of SRL of goat with different sources of mimosine are set out in Tables 14 to 18 and Fig. 4., summarised data in Table 19 and statistical analysis in Tables 20 and 21.

The data on percentage degradation of mimosine are detailed in Tables 22 to 26 and Fig. 5., summarised data in Table 27 and statistical analysis in Table 28 and 29.

The quadratic relationship between mimosine concentration and incubation time is given in Table 30 and Fig. 6.

The data on rate of disappearance of mimosine are set out in Table 31.

4.4. Effect of mimosine on rumen microbes.

VFA concentrations at different time intervals of incubation of SRL of goat with different sources of mimosine are detailed in Tables 32 to 37 and Fig. 7., summarised data in Table 38 and statistical analysis in Tables 39 and 40.

The ammonia concentrations at different time intervals of incubation of SRL of goat with different sources of mimosine are presented in Tables 41 to 46 and Fig. 8. summarised data in Table 47 and statistical analysis in Tables 48 and 49.

Table 3. Proximate chemical composition of immature leucaena leaves

Sample No.	Dry matter (%)	Per cent on dry matter basis				
		Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract
1	27.65	30.95	5.88	9.29	9.81	44.07
2	28.15	30.12	6.13	9.55	9.33	44.87
3	28.47	30.49	5.76	9.69	9.31	44.75
4	31.13	31.67	5.49	9.34	9.11	44.39
5	30.18	31.74	5.63	9.44	9.42	43.77
6	30.80	30.58	5.45	9.77	9.75	44.45
7	29.72	30.45	5.23	9.56	9.42	45.34
8	29.37	30.62	5.44	9.67	9.33	44.94
9	29.18	30.58	5.58	9.48	9.71	44.65
Mean ±S.E	29.40 ±0.39	30.80 ±0.19	5.62 ±0.08	9.53 ±0.05	9.46 ±0.08	44.58 ±0.16

Table 4. Proximate chemical composition of mature leucaena leaves

Sample No.	Dry matter (%)	Per cent on dry matter basis				
		Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract
1	33.53	25.52	4.32	12.76	11.51	45.89
2	35.51	26.08	4.49	12.70	11.56	45.17
3	34.46	25.18	4.14	12.73	11.23	46.72
4	35.52	25.67	4.12	12.30	11.26	46.65
5	36.43	25.94	4.37	11.25	10.87	47.57
6	35.02	26.69	3.99	11.96	10.85	46.51
7	36.12	25.62	4.23	12.03	10.96	47.16
8	35.68	25.43	4.34	12.12	11.03	47.08
9	35.79	25.58	4.41	12.26	10.95	46.80
Mean ±S.E	35.01 ±0.43	25.75 ±0.15	4.27 ±0.05	12.23 ±0.16	11.13 ±0.09	46.61 ±0.24

Table 5. Proximate chemical composition of tender stems of leucaena

Sample No.	Dry matter (%)	Per cent on dry matter basis				
		Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract
1	38.71	9.58	3.25	35.80	6.07	45.30
2	38.51	10.12	3.24	36.17	6.33	44.14
3	38.58	10.05	3.31	33.94	6.22	46.48
4	37.07	10.99	3.39	36.52	6.82	42.28
5	37.44	10.68	3.15	36.01	7.13	43.03
6	37.91	10.01	3.26	35.74	6.40	44.59
7	38.34	10.13	3.47	36.12	6.92	43.36
8	38.12	10.76	3.33	36.28	7.04	42.59
9	37.98	10.61	3.52	35.96	6.87	43.04
Mean ±S.E	38.07 ±0.18	10.32 ±0.15	3.32 ±0.04	35.83 ±0.25	6.64 ±0.13	43.87 ±0.46

Table 6. Proximate chemical composition of leucaena seeds

Sample No.	Dry matter (%)	Per cent on dry matter basis				
		Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract
1	92.34	26.34	8.53	13.92	4.97	46.31
2	92.21	25.82	8.54	14.32	4.57	46.75
3	92.18	26.81	8.25	14.55	4.69	45.70
4	92.15	27.25	8.16	14.46	4.23	45.90
5	92.19	25.24	8.28	14.37	4.48	47.63
6	92.10	26.01	8.21	14.42	4.37	46.99
7	91.96	25.78	8.18	13.96	4.31	47.77
8	92.34	26.31	8.26	14.27	4.27	46.89
9	92.37	26.42	8.32	14.22	4.26	46.78
Mean ±S.E	92.20 ±0.04	26.22 ±0.20	8.30 ±0.05	14.27 ±0.07	4.46 ±0.08	46.75 ±0.23

Table 7. Summarised data on proximate chemical composition of different edible parts of leucaena

Sample	Dry matter (%)	Per cent on dry matter basis				
		Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract
Immature leaves	29.40±0.39	30.80±0.19	5.62±0.08	9.53±0.05	9.46±0.08	44.58±0.16
Mature leaves	35.01±0.43	25.75±0.15	4.27±0.05	12.23±0.16	11.13±0.09	46.61±0.24
Tender stems	38.07±0.18	10.32±0.15	3.32±0.04	35.83±0.25	6.64±0.13	43.87±0.46
Seeds	92.20±0.04	26.22±0.20	8.30±0.05	14.27±0.07	4.46±0.08	46.75±0.23

Table 8. Mimosine content of different edible parts of leucaena on dry matter basis during the month of May.

Sample No.	Immature leaves (%)	Mature leaves (%)	Tender stems (%)	Seeds (%)
1	12.31	4.92	4.03	10.94
2	11.86	4.80	3.80	10.50
3	12.09	4.92	4.03	10.50
4	12.31	4.80	4.03	10.94
5	12.09	4.92	3.68	10.72
6	11.86	4.86	3.80	10.50
7	12.09	4.92	3.91	10.50
8	12.09	4.86	4.03	11.17
9	12.31	4.92	3.80	10.72
10	12.09	4.92	3.91	10.50
Mean ±S.E	12.11 ±0.05	4.89 ±0.02	3.90 ±0.04	10.70 ±0.08

Table 9. Mimosine content of different edible parts of leucaena on dry matter basis during the month of June.

Sample No.	Immature leaves (%)	Mature leaves (%)	Tender stems (%)	Seeds (%)
1	11.86	5.31	3.68	10.51
2	11.41	5.30	3.62	10.61
3	11.86	5.24	3.50	10.38
4	11.74	5.02	3.62	10.26
5	11.52	5.13	3.73	10.61
6	11.63	5.13	3.68	10.38
7	11.74	5.34	3.62	10.51
8	11.86	5.18	3.44	10.17
9	11.52	5.30	3.73	10.38
10	11.41	5.34	3.62	10.61
Mean ±S.E	11.66 ±0.06	5.23 ±0.03	3.62 ±0.03	10.44 ±0.05

Table 10. Mimosine content of different edible parts of leucaena on dry matter basis during the month of July.

Sample No.	Immature leaves (%)	Mature leaves (%)	Tender stems (%)	Seeds (%)
1	10.07	4.92	3.68	9.60
2	10.07	5.03	3.80	9.38
3	9.85	4.80	3.80	9.60
4	10.07	4.92	3.68	9.60
5	9.62	4.69	3.68	9.38
6	9.85	5.03	3.68	9.60
7	10.07	4.92	3.80	9.38
8	9.85	4.92	3.68	9.60
9	10.07	5.03	3.80	9.38
10	10.07	4.92	3.68	9.60
Mean ±S.E	9.96 ±0.05	4.92 ±0.03	3.73 ±0.02	9.51 ±0.04

Table 11. Summarised data on the mimosine content (%) of different edible parts of leucaena.

Months	Immature leaves	Mature leaves	Tender stems	Seeds
May	12.11±0.05	4.89±0.02	3.90±0.04	10.70±0.08
June	11.66±0.06	5.23±0.03	3.62±0.03	10.44±0.05
July	9.96±0.05	4.92±0.03	3.73±0.02	9.51±0.04

Table 12. Statistical analysis of data on mimosine content of leucaena during May, June and July.

Sample	't' values		
	May Vs June	May Vs July	June Vs July
Immature leaves	5.8489**	29.7293**	22.2732**
Mature leaves	9.0730**	0.9065(NS)	6.4284**
Tender stems	5.5699**	3.8830**	2.9403**
Seeds	2.8163*	13.9427**	15.4160**

* Significant at 5 per cent level
** Significant at 1 per cent level
NS Not significant

Table 13. Statistical analysis of data on mimosine content of different edible parts of leucaea

Months	't' values					
	Immature leaves Vs Mature leaves	Immature leaves Vs Tender stems	Immature leaves Vs Seeds	Mature leaves Vs Tender stems	Mature leaves Vs Seeds	Tender stems Vs Seeds
May	131.9181**	124.1862**	15.1189*	22.6481**	73.7232**	78.0211**
June	95.8240**	124.3015**	16.1105**	35.3881**	87.4726**	119.9861**
July	83.5551**	116.2428**	7.2766**	30.3767**	93.4157**	142.2915**

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 14. *In vitro* degradation of pure mimosine by strained rumen liquor (SRL) of goat

Sample No.	Added (pure) mimosine (mg/100 ml SRL)	Mimosine concentration (mg/100 ml SRL) 0 hr	12 hr	24 hr	36 hr	48 hr	% loss at 0hr
1	37.50	35.34	27.30	25.68	27.20	24.64	5.76
2	,,	36.42	32.10	24.60	26.76	25.68	2.88
3	,,	34.78	32.10	28.88	25.66	25.66	7.25
4	,,	35.86	31.04	24.58	23.50	23.50	4.37
5	,,	34.26	31.04	27.28	25.66	25.14	8.64
6	,,	35.34	28.90	26.74	22.46	21.40	5.76
7	,,	35.86	29.42	28.36	24.62	24.08	4.37
8	,,	34.80	28.90	26.74	24.62	23.54	7.20
9	,,	35.34	35.34	22.16	22.46	23.56	5.76
10	,,	35.34	30.52	25.66	25.12	24.58	5.76
11	,,	33.72	29.45	26.76	24.62	22.46	10.08
12	,,	34.80	28.90	26.74	24.62	23.54	7.20
Mean ±S.E	37.50	35.16 ±0.21	30.41 ±0.61	26.18 ±0.52	24.78 ±0.43	23.98 ±0.37	6.17 ±0.58

Table 15. *In vitro* degradation of mimosine of immature leucaena leaves by SRL of goat

Sample No.	Added (immature leaf) mimosine (mg/100 ml SRL)	Mimosine concentration (mg/100 ml SRL) 0 hr	12 hr	24 hr	36 hr	48 hr	% loss at 0hr
1	37.5	36.42	33.18	25.68	24.60	22.48	2.88
2	,,	34.26	29.22	27.82	24.60	23.56	8.64
3	,,	35.86	32.10	27.28	23.52	23.52	4.37
4	,,	34.78	28.34	26.20	25.12	24.60	7.25
5	,,	35.34	28.90	25.16	23.52	20.32	5.76
6	,,	36.40	29.96	25.68	23.54	23.00	2.93
7	,,	34.80	29.42	27.28	23.00	23.00	7.20
8	,,	35.86	30.50	25.68	23.54	22.46	4.37
9	,,	35.34	32.10	26.76	25.68	23.56	5.76
10	,,	35.34	31.56	25.72	25.64	25.64	5.76
11	,,	34.80	30.50	26.20	23.52	22.46	7.20
12	,,	34.80	28.36	26.76	23.02	23.02	7.20
Mean ±S.E	37.50	35.33 ±0.19	30.35 ±0.46	26.44 ±0.23	24.11 ±0.28	23.14 ±0.37	5.78 ±0.53

Table 16. *In vitro* degradation of mimosine of mature leucaena leaves by SRL of goat

Sample No.	Added (mature leaf) mimosine (mg/100 ml SRL)	Mimosine concentration (mg/100 ml SRL) 0 hr	12 hr	24 hr	36 hr	48 hr	% loss at 0 hr
1	37.50	36.42	32.10	27.20	24.68	22.48	2.88
2	,,	35.34	33.18	26.76	24.60	21.40	5.76
3	,,	33.70	28.34	24.60	23.52	23.52	10.13
4	,,	36.38	28.88	25.14	24.06	22.46	2.98
5	,,	35.86	25.90	25.68	23.60	20.84	4.37
6	,,	34.80	27.82	26.22	23.00	21.92	7.20
7	,,	33.72	28.34	25.66	20.86	20.86	10.08
8	,,	35.86	29.42	27.80	24.04	21.94	4.37
9	,,	36.42	32.10	25.68	24.60	23.56	2.88
10	,,	34.24	29.42	25.66	23.52	23.52	8.68
11	,,	35.34	28.36	25.68	22.46	22.46	5.76
12	,,	35.86	28.36	24.08	21.94	21.40	4.37
Mean ±S.E	37.50	35.33 ±0.28	29.61 ±0.52	25.85 ±0.30	23.41 ±0.34	22.20 ±0.28	5.79 ±0.77

Table 17. *In vitro* degradation of mimosine of tender leucaena stems by SRL of goat

Sample No.	Mimosine concentration (mg/100 ml SRL)						
	Added (tender stem) mimosine (mg/100 ml SRL)	0 hr	12 hr	24 hr	36 hr	48 hr	% loss at 0hr
1	37.50	34.26	29.22	27.82	27.20	25.60	8.64
2	,,	35.34	32.10	28.20	26.76	24.64	5.76
3	,,	36.38	32.62	26.74	25.66	23.52	2.98
4	,,	35.34	30.52	27.30	24.62	24.62	5.76
5	,,	33.72	27.82	27.28	22.46	20.32	10.08
6	,,	35.34	28.88	25.14	21.38	20.84	5.76
7	,,	36.38	30.48	28.34	25.66	23.52	2.98
8	,,	35.34	29.98	27.28	25.14	21.92	5.76
9	,,	35.34	29.22	27.20	26.76	25.60	5.76
10	,,	35.34	28.90	26.76	24.08	22.46	5.76
11	,,	34.80	28.36	27.28	23.52	23.00	7.20
12	,,	36.38	30.48	27.80	22.46	21.38	2.98
Mean ± S.E	37.50	35.33 ±0.24	29.88 ±0.42	27.26 ±0.24	24.64 ±0.56	23.12 ±0.52	5.79 ±0.63

Table 18. *In vitro* degradation of mimosine of leucaena seeds by SRL of goat

Sample No.	Added (seed) mimosine (mg/100 ml SRL)	Mimosine concentration (mg/100 ml SRL) 0 hr	12 hr	24 hr	36 hr	48 hr	% loss at 0hr
1	37.50	35.34	29.22	26.76	25.60	24.64	5.76
2	,,	34.26	28.20	26.76	25.60	24.60	8.64
3	,,	35.84	29.40	27.26	23.50	23.50	4.42
4	,,	34.24	28.88	25.66	24.04	22.42	8.69
5	,,	34.80	30.48	25.66	24.60	24.60	7.20
6	,,	34.80	28.88	27.28	24.60	24.60	7.20
7	,,	34.86	29.42	26.22	23.54	23.54	4.37
8	,,	34.80	28.90	25.68	24.06	22.44	7.20
9	,,	35.34	29.22	25.60	24.60	23.56	5.76
10	,,	34.24	27.80	23.52	22.42	20.28	8.69
11	,,	35.34	29.98	25.16	22.98	22.98	5.76
12	,,	35.86	28.90	27.82	25.14	22.98	4.37
Mean ±S.E	37.50	35.06 ±0.17	29.11 ±0.21	26.12 ±0.84	24.22 ±0.29	23.35 ±0.37	6.50 ±0.48

Table 19. Summarised data on *in vitro* degradation of mimosine by SRL of goat

Sample	Added mimosine (mg/100 ml SRL)	0 hr	12 hr	24 hr	36 hr	48 hr	% loss at 0 hr
Pure mimosine (T_1)	37.50	35.16±0.21	30.41±0.61	26.18±0.52	24.78±0.43	23.98±0.37	6.17±0.58
Immature leaves (T_2)	37.50	35.33±0.19	30.35±0.46.	26.44±0.23	24.11±0.28	23.14±0.37	5.78±0.53
Mature leaves (T_3)	37.50	35.33±0.28	29.61±0.52	25.85±0.30	23.41±0.34	22.20±0.28	5.79±0.77
Tender stem (T_4)	37.50	35.33±0.24	29.88±0.42	27.26±0.24	24.64±0.56	23.12±0.52	5.79±0.63
Seeds (T_5)	37.50	35.06±0.17	29.11±0.21	26.12±0.84	24.22±0.29	23.35±0.37	6.50±0.48

Table 20. Statistical analysis of data on *in vitro* degradation of mimosine by SRL of goat at different intervals of time.

Sample	't' values									
	0Vs12 hr	0Vs24 hr	0Vs36 hr	0Vs48 hr	12Vs24 hr	12Vs36 hr	12Vs48 hr	24Vs36 hr	24Vs48 hr	36VS48
T_1	7.7550**	14.0894**	22.0710**	25.5782**	4.2581**	6.6611**	10.4132**	2.4463*	3.6031**	2.8327**
T_2	13.4048**	23.3645**	32.1027**	26.9753**	7.5212**	14.1865**	13.2017**	6.7792**	10.4826**	3.2563**
T_3	12.1724**	36.2879**	39.5272**	31.6781**	8.2132**	16.8206**	13.3816**	7.3691**	8.3196**	3.6336**
T_4	17.8122**	24.7658**	18.4193**	22.0261**	6.2906**	10.1727**	12.6256**	5.7302**	8.8975**	5.8296**
T_5	30.0914**	30.7127**	32.2148**	31.8619**	8.1253**	13.9155**	17.7250**	7.3389**	9.8010**	3.5156**

T_1 - Pure mimosine, T_2 - Immature leaves, T_3 - Mature leaves, T_4 - Tender stems, T_5 - Seeds

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 21. Statistical analysis of data on *in vitro* degradation of mimosine of different edible parts of leucaena.

Time hr	't' values									
	T_1VsT_2	T_1VsT_3	T_1VsT_4	T_1VsT_5	T_2VsT_3	T_2VsT_4	T_2VsT_5	T_3VsT_4	T_3VsT_5	T_4VsT_5
0	0.5579 NS	0.4682	0.6589	0.2933	0.0143	0.0097	1.0910	0.0036	0.7144	1.1938
12	0.0854	1.2328	0.7966	1.9918	1.3384	0.7205	2.4008*	0.4560	0.8029	1.4687
24	0.4325	0.5137	1.8220	0.1237	1.3516	3.1769**	0.8417	3.7520**	0.5497	2.7967*
36	1.2551	2.8038*	0.2445	1.2148	2.7981*	1.0802	0.2805	2.5023*	2.3235*	0.7389
48	1.6243	3.8894**	1.5885	1.2687	3.1011**	0.0331	0.3111	1.9734	2.0579	0.3707

T_1 - Pure mimosine, T_2 - Immature leaves, T_3 Mature leaves T_4 - Tender stem T_5 - Seeds

* Significant at 5 % level

** Significant at 1 % level

Table 22. Percentage *in vitro* degradation of mimosine (pure) by SRL of goat.

Sample No.	12 hr	24 hr	36 hr	48 hr
1	23.00	27.33	23.03	30.28
2	11.86	32.45	26.52	29.48
3	7.70	16.96	26.22	26.22
4	13.44	31.46	34.47	34.47
5	9.39	20.38	25.10	26.62
6	18.22	24.34	36.45	39.45
7	17.96	20.91	31.34	31.86
8	16.95	23.16	29.25	32.36
9	0.00	37.29	36.45	33.33
10	13.64	27.39	28.92	30.45
11	12.69	20.64	26.99	33.39
12	16.95	23.16	29.25	32.36
Mean +S.E	13.48 ±1.72	25.45 ±1.7	29.49 ±1.27	31.69 ±1.02

Table 23. Percentage *in vitro* degradation of mimosine (Immature leucaena leaves) by SRL of goat

Sample No.	12 hr	24 hr	36 hr	48 hr
1	8.90	29.49	32.45	38.28
2	14.71	18.80	28.20	31.23
3	10.48	23.93	34.41	34.41
4	18.52	24.67	27.78	29.27
5	18.22	28.81	33.45	42.50
6	17.70	29.45	35.33	36.81
7	15.46	21.61	33.91	33.91
8	14.95	28.39	34.36	37.37
9	9.17	24.38	27.33	33.33
10	10.70	24.39	27.45	27.45
11	12.10	24.71	32.41	35.46
12	18.51	23.10	33.85	33.85
Mean ±S.E	14.12 ±1.07	25.14 ±0.96	31.74 ±0.90	34.49 ±1.18

Table 24. Percentage *in vitro* degradation of mimosine (Mature leucaena leaves) by SRL of goat.

Sample No.	12 hr	24 hr	36 hr	48 hr
1	11.86	25.32	32.24	38.28
2	6.11	24.36	30.39	39.45
3	15.91	27.00	30.21	30.21
4	20.62	30.90	33.86	38.26
5	19.41	28.39	34.19	41.89
6	20.06	24.66	66.09	37.01
7	15.95	23.90	38.14	38.14
8	17.96	22.48	32.96	38.82
9	11.86	29.49	32.45	35.31
10	14.08	25.06	31.31	31.31
11	19.75	27.33	36.45	36.45
12	20.91	32.85	38.82	40.32
Mean ±S.E	16.21 ±1.30	26.81 ±0.89	36.43 ±2.81	37.12 ±0.99

Table 25. Percentage *in vitro* degradation of mimosine (Tender leucaena stems) by SRL of goat

Sample No.	12 hr	24 hr	36 hr	48 hr
1	14.71	18.80	20.61	25.28
2	9.17	20.20	24.28	30.28
3	10.34	26.49	29.47	35.35
4	13.64	22.75	30.33	30.33
5	17.50	19.10	33.39	39.74
6	18.28	28.86	39.50	41.03
7	16.22	22.10	29.47	35.35
8	15.17	22.81	28.86	37.97
9	17.32	23.03	24.28	27.56
10	18.22	24.28	31.86	36.45
11	18.51	21.61	32.41	33.91
12	16.22	17.64	38.26	41.23
Mean ±S.E	15.44 ±0.88	22.31 ±0.68	30.22 ±1.60	34.54 ±1.50

Table 26. Percentage *in vitro* degradation of mimosine (Leucaena seeds) by SRL of goat.

Sample No.	12 hr	24 hr	36 hr	48 hr
1	17.32	24.28	27.56	31.38
2	17.69	21.89	25.28	28.20
3	17.97	23.94	34.43	34.43
4	15.65	25.06	29.79	34.52
5	12.41	26.26	29.31	29.31
6	17.01	21.61	29.31	29.31
7	17.96	26.88	34.36	34.36
8	16.95	26.21	30.86	35.52
9	17.31	27.56	30.39	33.33
10	18.81	31.31	34.52	40.77
11	15.17	28.81	34.97	34.97
12	19.41	22.42	29.89	35.92
Mean ±S.E	16.97 ±0.53	25.52 ±0.84	30.89 ±0.89	33.41 ±1.03

Table 27. Summarised data on percentage *in vitro* degradation of mimosine by SRL of goat

Sample No.	12 hr	24 hr	36 hr	48 hr
Pure mimosine	13.48±1.72	25.45±1.7	29.49±1.27	31.69±1.02
Immature leaves	14.12±1.07	25.14±0.96	31.74±0.90	34.49±1.18
Mature leaves	16.21±1.30	26.81±0.89	36.43±2.81	37.12±0.99
Tender stem	15.44±0.88	22.31±0.68	30.22±1.60	34.54±1.50
Seeds	16.97±0.53	25.52±0.84	30.89±0.89	33.41±1.03

Table 28. Statistical analysis of data on percentage *in vitro* degradation of mimosine by SRL of goat at different intervals of time.

Sample	't' values					
	12Vs24	12Vs36	12Vs48	24Vs36	24Vs48	36Vs48
Pure mimosine (T_1)	4.3065**	6.7215**	10.3425**	2.4919*	3.6258**	2.6863*
Immature leaves (T_2)	7.7431**	14.8879**	13.7986**	6.6812**	10.3001**	3.2629**
Mature leaves (T_3)	8.4051**	7.9494**	13.8959**	3.1579**	8.3491**	0.2428NS
Tender stem (T_4)	5.5054**	10.4605**	13.0599**	4.9169**	7.6300**	5.8301**
Seeds (T_5)	8.0648**	14.1286**	17.4594**	7.5461**	9.9894**	3.5167**

* Significant at 5% level
** Significant at 1% level
NS Not Significant

Table 29. Statistical analysis of data on percentage *in vitro* degradation of mimosine of different edible parts of leucaena by SRL of goat

Time (hr)	't' values									
	T1VsT2	T1VsT3	T1VsT4	T1VsT5	T2VsT3	T2VsT4	T2VsT5	T3VsT4	T3VsT5	T4VsT5
12	0.3556	1.3956	1.0436	2.0564	1.8763	0.9854	2.1469	0.6637	0.4879	1.3523
24	0.1496	0.7537	1.5596	0.0334	1.2042	2.3143*	0.3028	3.0061**	0.9989	2.5868*
36	1.3107	[illegible]**	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]**	[illegible]	[illegible]
48	1.6700	4.0508**	1.6477	1.1784	2.3539*	0.0303	0.5531	1.5102	2.0933	0.6779

T1 - Pure mimosine, T2 - Immature leaves, T3 - Mature leaves, T4 - Tender stem, T5 - Seeds

* Significant at 5% level

** Significatn at 1% level

Table 30. Quadratic equations representing the relationship between mimosine concentration and incubation time.

Treatments	Equation	Coefficient of determination (R^2)
Pure mimosine	$y = 352.33-4.89x+0.05x^2$	0.9966
Immature leaves	$y = 353.69-4.84x+0.05x^2$	0.9998
Mature leaves	$y = 352.49-5.17x+0.05x^2$	0.9995
Tender stems	$y = 351.01-4.34x+0.04x^2$	0.9944
Seeds	$y = 348.41-5.04x+0.06x^2$	0.9953

Table 31. Rate of disappearance of mimosine at different time intervals ($\mu g.ml^{-1}.hr^{-1}$).

Sample	0 to 12 hr	12 to 24 hr	24 to 36 hr	36 to 48 hr	0 to 48 hr
Pure mimosine	3.95	3.52	1.16	0.67	2.33
Immature leaves	4.15	3.26	1.94	0.81	2.54
Mature leaves	4.77	3.13	2.03	1.00	2.74
Tender stems	4.54	2.18	2.18	1.26	2.54
Seeds	4.96	2.49	1.58	0.73	2.44

Table 32. VFA concentration of SRL of goat at different time intervals of incubation without mimosine

(m. moles / 100 ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	9.17	10.58	10.34	10.05	9.34
2	8.76	10.29	9.70	9.20	8.93
3	9.34	10.05	9.76	9.64	9.46
4	8.99	10.23	9.64	9.58	9.11
5	9.46	10.46	10.34	9.87	9.64
6	9.58	10.49	10.05	9.81	9.64
7	9.58	10.49	9.81	9.81	9.58
Mean ±S.E	9.26 ±0.12	10.37 ±0.07	9.95 ±0.11	9.71 ±0.10	9.38 ±0.10

Table 33. VFA concentration of SRL of goat at different time intervals of incubation with mimosine (pure)

(m.moles/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	9.26	10.46	10.26	9.93	9.34
2	8.93	10.20	9.64	9.34	8.99
3	9.26	9.96	9.64	9.55	9.34
4	8.96	10.20	9.70	9.61	9.17
5	9.52	10.40	10.23	9.81	9.58
6	9.52	10.37	10.17	9.87	9.37
7	9.52	10.52	9.84	9.76	9.49
Mean ±S.E	9.28 ±0.09	10.30 ±0.07	9.93 ±0.10	9.70 ±0.07	9.37 ±0.08

Table 34. VFA concentration of SRL of goat at different time intervals of incubation with mimosine (immature leucaena leaves)

(m.moles/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	9.17	10.52	10.20	9.90	9.26
2	8.90	10.23	9.79	9.17	8.87
3	9.34	10.11	9.67	9.58	9.34
4	9.05	10.11	9.81	9.46	9.05
5	9.34	10.46	10.29	9.76	9.46
6	9.33	10.40	10.23	9.76	9.46
7	9.46	10.43	9.76	9.84	9.52
Mean $\pm$S.E	9.23 $\pm$0.07	10.32 $\pm$0.06	9.96 $\pm$0.10	9.64 $\pm$0.10	9.28 $\pm$0.10

Table 35. VFA concentration of SRL of goat at different time intervals of incubation with mimosine (mature leucaena leaves)

(m.moles/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	9.23	10.67	10.34	10.11	9.29
2	8.73	10.17	9.64	9.23	8.99
3	9.46	10.17	9.76	9.67	9.37
4	8.87	10.17	9.70	9.52	9.11
5	9.58	10.29	10.34	9.70	9.52
6	9.31	10.46	10.11	9.87	9.52
7	9.34	10.46	9.73	9.76	9.58
Mean ±S.E	9.21 ±0.12	10.34 ±0.07	9.95 ±0.11	9.69 ±0.10	9.34 ±0.08

Table 36. VFA concentration of SRL goat at different time intervals of incubation with mimosine (tender leucaena stem).

(m.moles/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	9.14	10.55	10.31	10.14	9.26
2	8.93	10.31	9.73	9.11	9.02
3	9.29	10.05	9.70	9.64	9.29
4	9.11	10.23	9.84	9.58	9.08
5	9.52	10.17	10.29	9.70	9.67
6	9.58	10.52	10.20	9.70	9.55
7	9.52	10.34	9.87	9.76	9.55
Mean ±S.E	9.30 ±0.09	10.31 ±0.07	9.99 ±0.10	9.66 ±0.11	9.35 ±0.09

Table 37. VFA concentration of SRL of goat at different time intervals of incubation with mimosine (leucaena seeds)

(m.moles/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	9.17	10.49	10.29	9.93	9.37
2	8.76	10.17	9.58	9.05	8.93
3	9.37	10.14	9.79	9.52	9.29
4	8.87	10.05	9.87	9.70	9.29
5	9.61	10.37	10.23	9.81	9.52
6	9.64	10.55	10.23	9.76	9.52
7	9.55	10.46	9.93	9.71	9.49
Mean ±S.E	9.28 ±0.13	10.32 ±0.07	9.99 ±0.10	9.64 ±0.10	9.34 ±0.08

Table 38. Summarised data on VFA concentration of rumen liquor incubated with different edible parts of leucaena.

(m.moles/100 ml R.L)

Sample	0 hr	12 hr	24 hr	36 hr	48 hr
Control (without mimosine)	9.26±0.12	10.37±0.07	9.95±0.11	9.71±0.10	9.38±0.10
Pure mimosine	9.28±0.09	10.30±0.07	9.93±0.10	9.70±0.07	9.37±0.08
Immature leaves	9.23+0.07	10.32+0.06	9.96+0.10	9.64+0.10	9.28+0.10
Mature leaves	9.21±0.12	10.34±0.07	9.95±0.11	9.69±0.10	9.34±0.08
Tender stems	9.30±0.09	10.31±0.07	9.99±0.10	9.66±0.11	9.35±0.09
Seeds	9.28±0.13	10.32±0.07	9.99±0.10	9.64±0.10	9.34±0.08

Table 39. Statistical analysis of data on VFA concentration of rumen liquor incubated with different edible parts of leucaena

Time (hr)	't' values														
	CVsT1	CVsT2	CVsT3	CVsT4	CVsT5	T1VsT2	T1VsT3	T1VsT4	T1VsT5	T2VsT3	T2VsT4	T2VsT5	T3VsT4	T3VsT5	T4VsT5
0	0.3588	0.8387	0.8341	0.8941	0.4108	1.2849	1.1128	0.5601	0.0000	0.1668	1.7411	0.7689	1.1887	1.1232	0.3489
12	3.3577**	2.0913	0.7236	1.3448	1.3950	0.6559	0.8077	0.1479	0.3648	0.4747	0.2304	0.1382	0.7317	0.6122	0.1614
24	0.6749	0.3152	0.1667	1.1059	0.7424	1.2395	0.6117	4.4963**	2.0383	0.4383	1.0982	0.4946	1.3539	0.9267	0.0927
36	0.3670	3.0177*	0.4487	1.4542	2.0099	1.6344	0.0329	0.5965	1.2314	1.4769	0.4961	0.0299	1.0498	0.9920	0.4591
48	0.6197	5.0823**	1.7589	1.2501	0.9098	2.8814*	0.9829	0.7375	0.7557	5.2915**	1.9284	1.7769	0.2033	0.1166	0.0306

C - Control, T1 - Pure mimosine, T2 - Immature leaves, T3 - Mature leaves, T4 - Tender stems, T5 - Seeds

* Significant at 5% level

** Significant at 1% level

Table 40. Statistical analysis of data on VFA concentration of SRL of goat at different intervals of time

Sample	't' values									
	0Vs12 hr	0Vs24 hr	0Vs36 hr	0Vs48 hr	12Vs24 hr	12Vs36 hr	12Vs48 hr	24Vs36 hr	24Vs48 hr	36Vs48 hr
C	9.7600**	5.4371**	5.0035**	4.6598**	5.3214**	8.2481**	9.7168**	3.2886**	5.4216**	4.3131**
T1	12.1939**	7.3885**	6.2192**	3.0474*	4.8808**	10.3549**	11.1341**	4.3466**	7.0549**	5.9980**
T2	14.3765**	6.5307**	6.8327**	2.1956*	5.4047**	10.4131**	13.4487**	3.4836**	6.4351**	7.0814**
T3	9.6157**	6.7301**	4.8786**	2.1547NS	4.3972**	11.8936**	12.0250**	3.0458*	5.5580**	4.2147**
T4	8.9028**	6.6985**	3.1465**	1.7041NS	3.7955**	6.0424**	8.5489**	3.7094**	7.1456**	2.8244*
T5	10.3721**	6.6165**	3.1247**	0.8145NS	4.9912**	7.4134**	15.2348**	6.8904**	10.7320**	5.3783**

C - Control, T1 - Pure mimosine, T2 - Immature leaves, T3 - Mature leaves, T4 - Tender stems, T5 - Seeds

* Significant at 5% level
** Significant at 1% level
NS Not Significant

Table 41. Ammonia concentration of SRL of goat at different time intervals of incubation without mimosine

(mg/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	13.72	13.86	5.88	-	-
2	13.16	14.42	1.12	-	-
3	14.35	14.98	1.68	-	-
4	13.72	14.56	2.10	-	-
5	13.30	14.42	1.82	-	-
6	13.51	14.63	1.89	-	-
7	13.65	14.70	1.68	-	-
Mean ±S.E	13.63 ±0.14	14.51 ±0.13	2.13 ±0.60	-	-

Table 42. Ammonia concentration of SRL of goat at different time intervals of incubation with mimosine (pure)

(mg/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	13.93	13.58	10.92	13.30	11.97
2	13.30	14.56	10.08	10.29	10.78
3	14.14	15.12	13.72	11.06	11.48
4	13.86	14.84	12.18	11.62	11.76
5	13.02	14.56	12.04	11.06	11.69
6	13.72	14.49	11.90	11.41	11.41
7	13.44	14.56	11.76	11.48	10.92
Mean ±S.E	13.63 ±0.15	14.53 ±0.18	11.80 ±0.43	11.46 ±0.35	11.43 ±0.17

Table 43. Ammonia concentration of SRL of goat at different time intervals of incubation with mimosine (immature leucaena leaves)

(mg/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	13.72	13.72	12.72	11.48	11.48
2	13.09	14.28	10.92	10.36	10.50
3	14.85	15.68	13.44	11.34	11.69
4	14.14	14.56	12.46	11.76	11.34
5	13.58	14.49	11.76	11.90	11.76
6	13.86	14.77	11.62	10.92	10.25
7	13.79	14.70	12.04	11.41	10.78
Mean ±S.E	13.80 ±0.20	14.60 ±0.22	12.14 ±0.31	11.31 ±0.20	11.25 ±0.18

Table 44. Ammonia concentration of SRL of goat at different time intervals of incubation with mimosine (mature leucaena leaves)

(mg/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	13.79	14.14	13.16	11.34	11.13
2	13.58	14.63	9.94	10.36	10.29
3	14.35	15.54	13.16	11.41	11.34
4	14.28	14.14	12.95	12.18	11.55
5	13.23	14.56	11.90	11.83	11.62
6	13.65	14.49	12.18	11.48	11.13
7	13.86	14.63	12.25	10.92	10.64
Mean ±S.E	13.82 ±0.15	14.59 ±0.18	12.22 ±0.43	11.36 ±0.22	11.10 ±0.18

Table 45. Ammonia concentration of SRL of goat at different time intervals of incubation with mimosine (tender leucaena stems)

(mg/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	13.44	13.93	11.13	11.06	11.34
2	13.16	14.70	11.06	11.06	11.48
3	14.28	15.40	13.86	11.34	11.69
4	13.79	14.28	13.02	11.97	11.83
5	13.30	14.63	12.18	11.13	11.90
6	13.58	14.21	12.11	10.99	11.06
7	13.58	14.70	12.04	11.13	11.27
Mean ±S.E	13.59 ±0.14	14.54 ±0.18	12.20 ±0.40	11.24 ±0.13	11.51 ±0.12

Table 46. Ammonia concentration of SRL of goat at different time intervals of incubation with mimosine (leucaena seeds)

(mg/100ml R.L)

Sample No.	0 hr	12 hr	24 hr	36 hr	48 hr
1	14.14	14.14	11.34	10.99	10.78
2	13.51	14.56	11.48	10.99	11.41
3	14.28	15.26	14.14	11.48	11.62
4	13.58	14.56	12.53	11.76	11.76
5	13.37	14.28	12.32	11.34	11.69
6	13.65	14.21	12.18	11.13	10.85
7	13.44	14.77	12.32	10.99	10.99
Mean ±S.E	13.71 ±0.13	14.54 ±0.15	12.33 ±0.35	11.24 ±0.11	11.26 ±0.16

Table 47. Summarised data on ammonia concentration of rumen liquor incubated with different edible parts of leucaena.

(mg/100 ml R.L)

Sample	0 hr	12 hr	24 hr	36 hr	48 hr
Control	13.63±0.14	14.51±0.13	2.13±0.60	-	-
Pure mimosine	13.63±0.15	14.53±0.18	11.80±0.43	11.46±0.35	11.43±0.17
Immature leaves	13.80+0.20	14.60+0.22	12.14+0.31	11.31+0.20	11.25+0.18
Mature leaves	13.82±0.15	14.59±0.18	12.22±0.43	11.36±0.22	11.10±0.18
Tender stems	13.59±0.14	14.54±0.18	12.20±0.40	11.24±0.13	11.51±0.12
Seeds	13.71±0.13	14.54±0.15	12.33±0.35	11.24±0.11	11.26±0.16

Table 48. Statistical analysis of data on ammonia concentration of rumen liquor incubated with different edible parts of leucaena

Time (hr)	't' values														
	CVsT1	CVsT2	CVsT3	CVsT4	CVsT5	T1VsT2	T1VsT3	T1VsT4	T1VsT5	T2VsT3	T2VsT4	T2VsT5	T3VsT4	T3VsT5	T4VsT5
0	0.0000	0.9242	0.9156	0.1996	0.4051	0.9157	0.9034	0.1967	0.3992	0.1639	1.0990	0.6191	1.1308	0.5486	0.6213
12	0.2582	0.8274	0.6628	0.3504	0.3192	0.6070	0.3959	0.1628	0.0829	0.0849	0.3893	0.4435	0.6378	0.5008	0.1207
24	11.6051**	17.5807**	18.4919**	12.0297**	12.3341**	1.1612	1.2116	2.9897*	3.5438**	0.3878	0.2081	0.6861	0.0529	0.2710	1.1643
36	-	-	-	-	-	0.4739	0.2863	0.5932	0.5782	0.3762	0.3901	0.4219	0.6845	0.7907	0.0000
48	-	-	-	-	-	1.8904	3.3449**	0.4772	0.5909	2.0735	1.6633	0.2520	2.7054*	1.0857	3.1539**

C - Control, T1 - Pure mimosine, T2 - Immature leaves, T3 - Mature leaves T4 - Tender stems, T5 - Seeds

* Significant at 5% level
** Significant at 1% level

Table 49. Statistical analysis of data on ammonia concentration of SRL of goat at different intervals of time.

Sample	't' values									
	0Vs12	0Vs24	0Vs36	0Vs48	12Vs24	12Vs36	12Vs48	24Vs36	24Vs48	36Vs48
C	6.0109**	18.8920**	- **	- **	17.0834**	- **	- **	- **	- **	NS
T1	3.9506**	4.8097**	7.0158**	12.7371**	7.9846*	6.1660**	11.0270**	0.5982 NS	0.9131 NS	0.0777 NS
T2	4.9179**	10.4991**	11.6431**	15.0622**	8.2137**	11.1670**	13.4612**	3.1666**	3.8030**	0.4380 NS
T3	3.9727**	4.4485**	10.3432**	12.6729**	5.1792**	10.1921**	13.4297**	2.7096*	3.4186**	3.5714**
T4	6.0000**	5.4051**	17.1088**	12.6041**	7.7323**	15.2571**	17.3314**	3.0388*	2.0439 NS	2.4648*
T5	5.1019**	4.3280**	14.6567**	10.7886**	9.0984**	21.5574**	19.9787**	3.7512**	3.4378**	0.6030 NS

C - Control, T1 - Pure mimosine, T2 - Immature leaves, T3 - Mature leaves, T4 - Tender stems, T5 - Seeds

* Significant at 5% level
** Significant at 1% level
NS Not Significant

Fig. 3. Mimosine content of different edible parts of leucaena

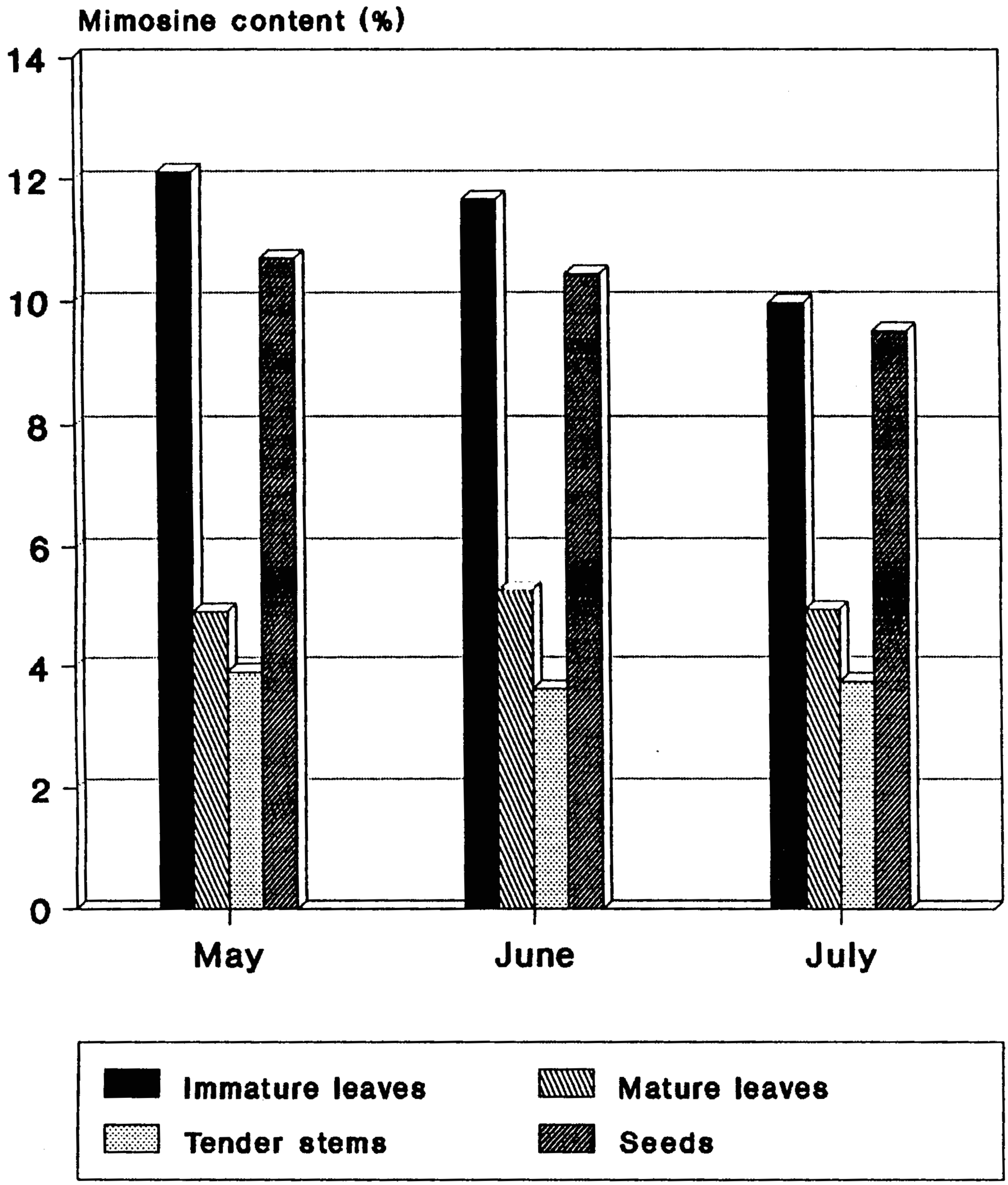


Fig.4 MIMOSINE CONCENTRATION OF SRL OF GOAT AT DIFFERENT PERIODS OF INCUBATION WITH DIFFERENT SOURCES OF MIMOSINE

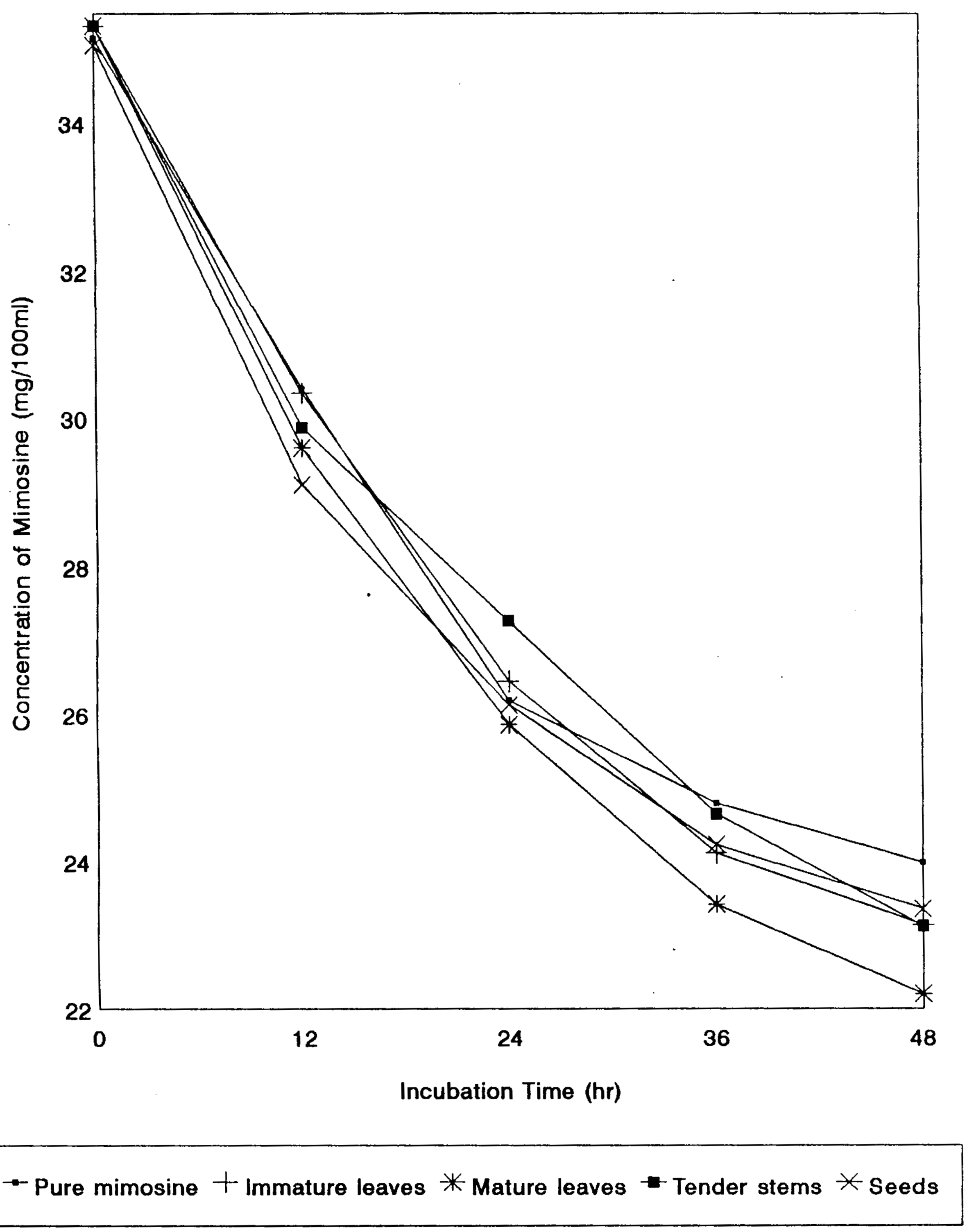


Fig.5 PERCENTAGE DEGRADATION OF MIMOSINE OF DIFFERENT SOURCES AT DIFFERENT PERIODS OF INCUBATION WITH SRL OF GOAT

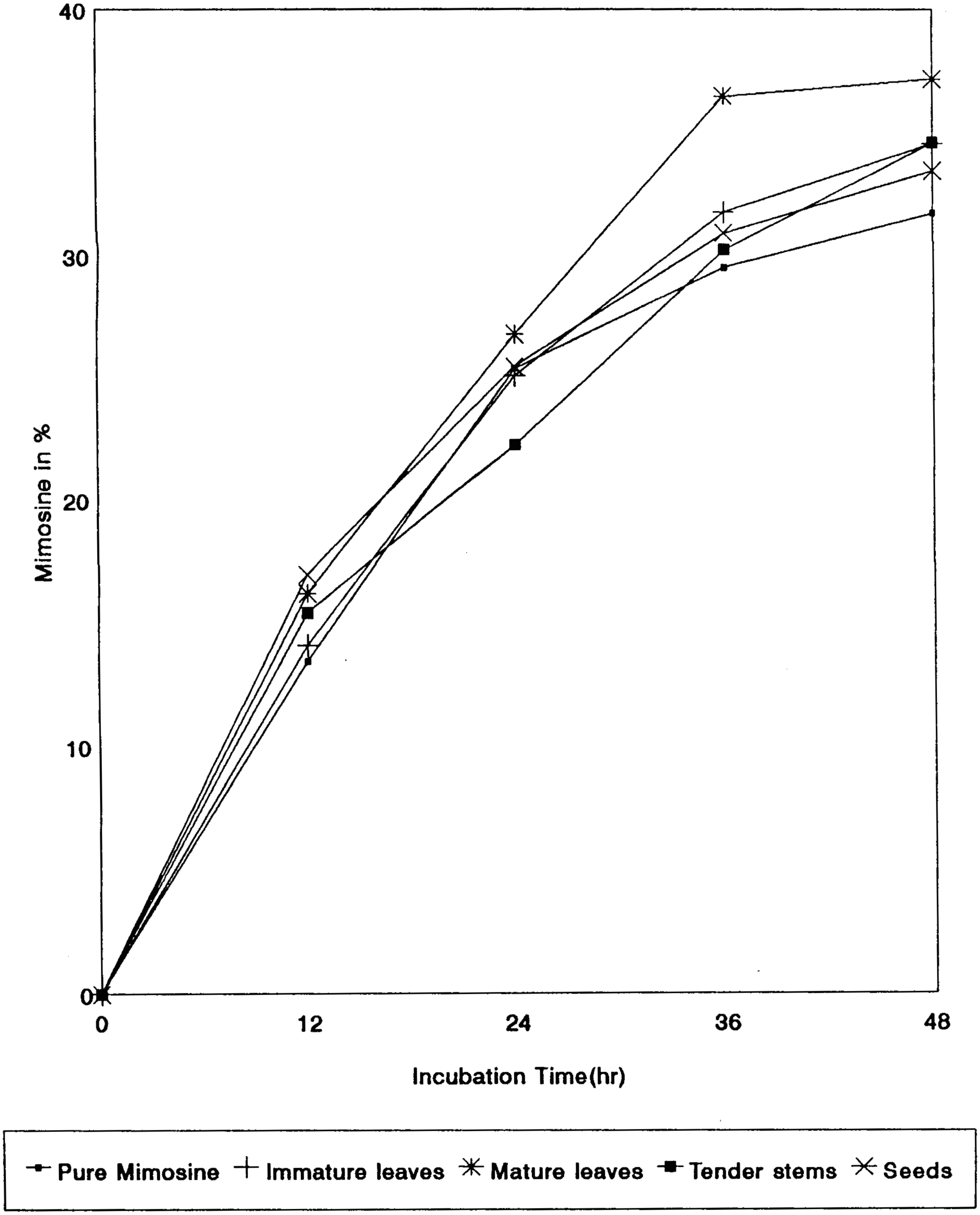


Fig.6 QUADRATIC RELATIONSHIP BETWEEN MIMOSINE CONCENTRATION AND INCUBATION TIME FOR DIFFERENT SOURCES OF MIMOSINE

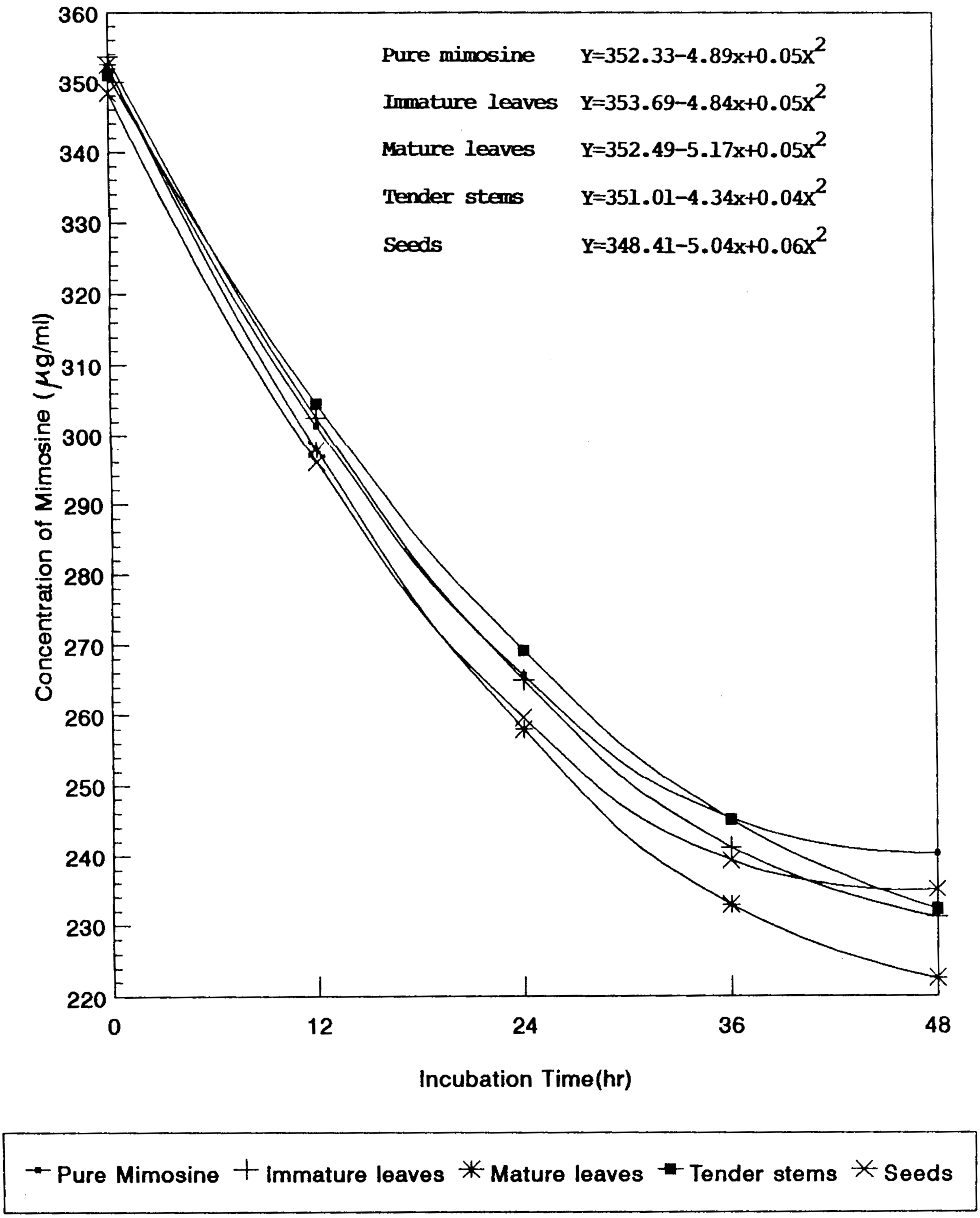


Fig.7 VFA CONCENTRATION OF SRL OF GOAT INCUBATED WITH DIFFERENT SOURCES OF MIMOSINE

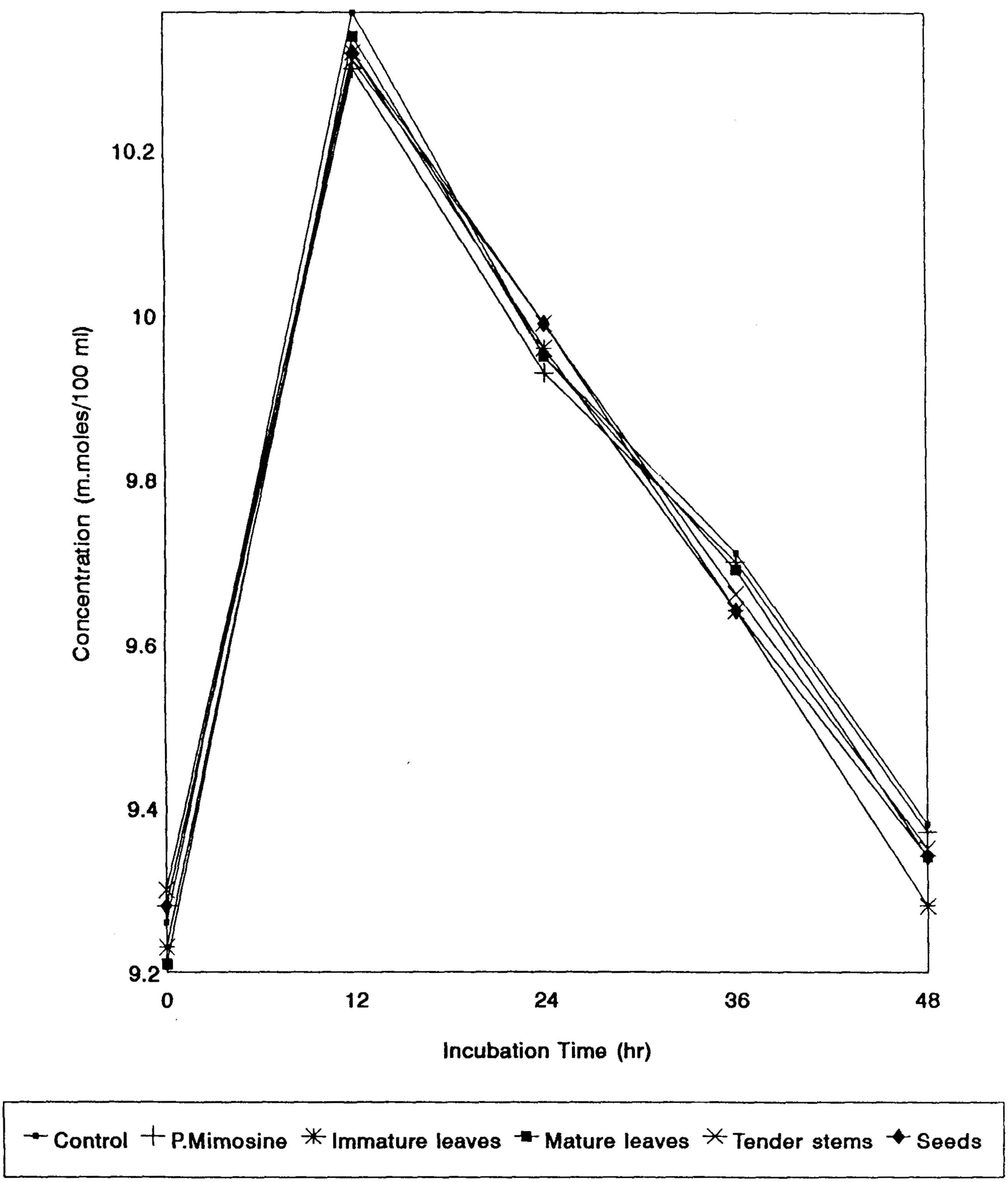
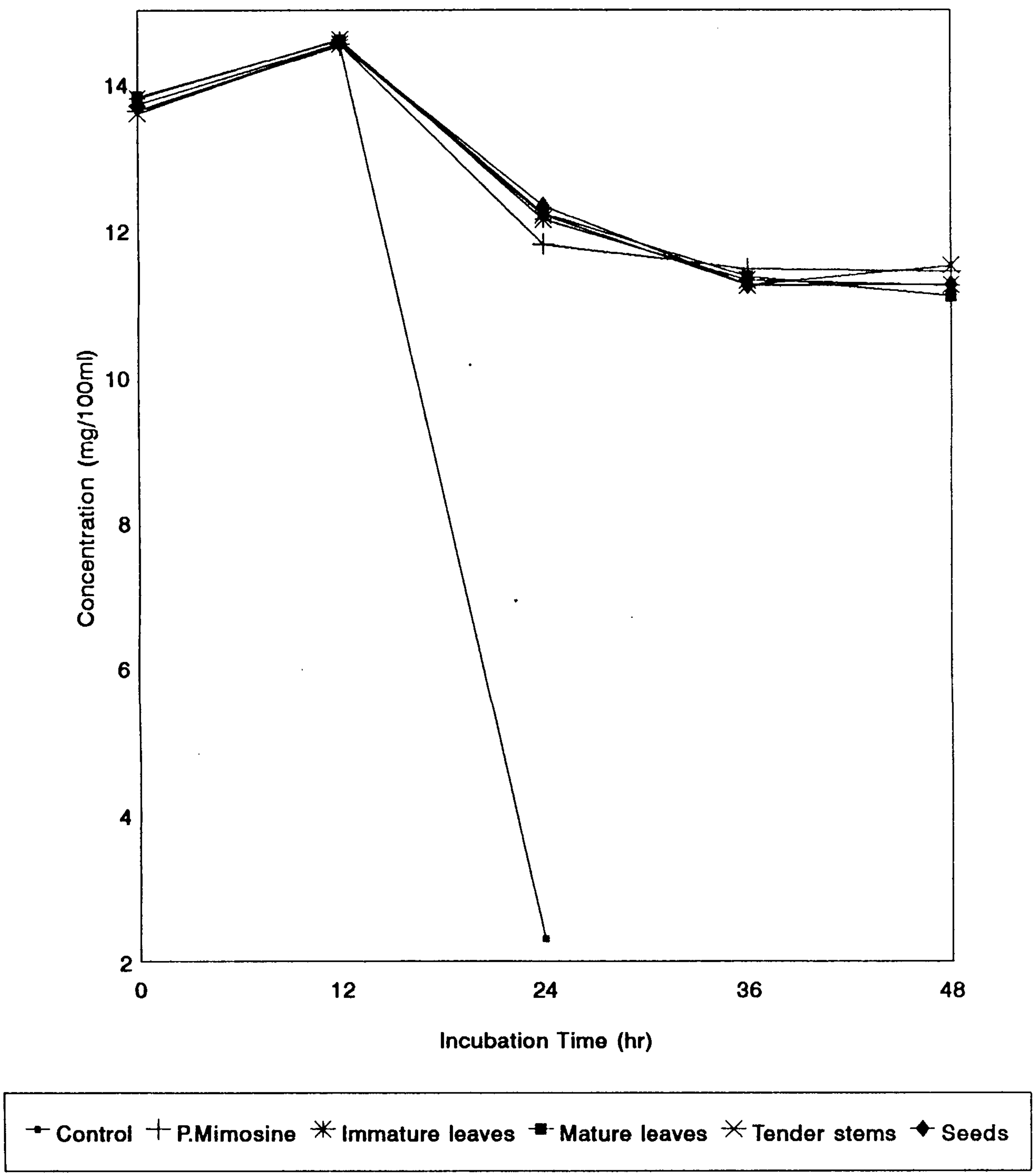


Fig.8 AMMONIA CONCENTRATION OF SRL OF GOAT INCUBATED WITH DIFFERENT SOURCES OF MIMOSINE



Discussion

DISCUSSION

Proximate composition of *Leucaena leucocephala*

It can be seen from the data on proximate composition of immature leaves, mature leaves, tender stems and seeds of *L. leucocephala*, given in Tables 3 to 6 respectively and from the summarised data in Table 7 that the different edible parts of the plant vary much in their proximate composition. While immature leaves contained only 29.40±0.39 per cent dry matter, mature leaves, tender stems and seeds contained on an average 35.01±0.43, 38.07±0.18 and 92.2±0.04 per cent respectively of dry matter.

It can be seen from Table 7 that immature leaves and seeds contained more crude protein viz., 30.80±0.19 and 26.22±0.20 per cent respectively than mature leaves which contained on an average 25.75±0.15 per cent of crude protein as previously reported (Hutton and Gray, 1959; Castillo *et al*., 1963 and Adeneye, 1979). Adeneye (1979), however, reported higher values of 34.4 to 40.1, 30.2 to 32.6 and 24.2 to 27.8 per cent respectively for the v-shaped semi-open, olive green open young and dark green mature leaves. While much lower crude protein contents of 24.42 and 19.82 respectively for immature and mature leaves were reported by Akbar and Gupta (1985), the protein content of 27.62 reported by them for seeds was essentially in keeping with the results obtained in the present study.

The seeds showed the highest crude fat content of 8.30±0.05 per cent on dry matter basis as against 5.62±0.08, 4.27±0.05 and 3.32±0.04 per cent in immature leaves, mature leaves and tender stem respectively. These results are essentially in unison with those of Akbar and Gupta (1985) who reported a fat content of 8.99 per cent in seeds. Adeneye (1979) also found that more of crude fat is stored in seeds (5.5%) than in any other edible parts of leucaena.

The tender stem contained the highest amount of 35.83±0.25 per cent crude fibre while immature leaves, mature leaves and seeds contained on an average 9.53±0.05, 12.23±0.16 and 14.27 ±0.07 per cent respectively of crude fibre on a dry matter basis. While the average crude fibre content of immature leaves and mature leaves in the present study are essentially in keeping with those reported by Akbar and Gupta (1985), they recorded a comparatively lower value of 11.03 per cent in respect of seeds as against 14.27± 0.07 per cent obtained in the present study. Adeneye (1979), however, reported a much higher crude fibre content of 35.8 per cent on dry matter basis in the brown seeds of <u>L. leucocephala</u> of Western Nigeria, while the values of 10.8 to 12.8 per cent in immature leaves and 14.4 per cent in mature leaves were not much different from the figures obtained in the present study.

The total ash content on dry matter basis varied from 4.46$\pm$0.08 per cent in seeds to 11.13$\pm$0.09 per cent in mature leaves with intermediate values of 9.46$\pm$0.08 and 6.64$\pm$0.13 per cent in the case of immature leaves and tender stems respectively. The mature leaves contained more ash than the immature leaves, as more and more minerals tend to accumulate in them as they mature. Presence of higher amount of total ash in leaves than in the seeds, noted in the present study, are in accordance with the results obtained by earlier workers (Adeneye, 1979 and Akbar and Gupta, 1985).

There was not much variation in respect of the nitrogen free extract content of different edible parts of L. leucocephala on dry matter basis, the average values being 44.58$\pm$0.16, 46.61$\pm$0.24, 43.87$\pm$0.46 and 46.75$\pm$0.23 per cent respectively for immature leaves, mature leaves, tender stems and seeds respectively. The nitrogen free extract values obtained for various edible parts of leucaena in the present study are essentially in keeping with those reported earlier (Adeneye, 1979 and Akbar and Gupta, 1985), even though Adeneye (1979) recorded a much lower nitrogen free extract value of 20.9 per cent in seeds on dry matter basis. However, Akbar and Gupta (1985) reported a much higher nitrogen free extract value of 57.35 per cent.

Mimosine content of different edible parts of leucaena

From the data on mimosine content of different edible parts of L. leucocephala for the months of May, June and July given in Tables 8 - 11 and Fig. 3 and from the statistical analysis presented in Table 13, it can be seen that there was much variation in the concentration of mimosine, in different edible parts and that the values varied between different months studied. The immature leaves contained the highest concentration of 12.11±0.05 per cent during the month of May, lesser concentration of 11.66±0.06 per cent during June and the least concentration of 9.96±0.05 per cent during the month of July, there being significant differences between the values pertaining to May and June ($P<0.01$), May and July ($P<0.01$) and June and July ($P<0.01$).

The mature subabul leaves contained on an average 4.89±0.02, 5.23±0.03 and 4.92±0.03 per cent mimosine during May, June and July respectively, there being significant differences between the values obtained in May and June ($P<0.01$) and those between June and July ($P<0.01$), even though there was no significant difference between the values obtained in May and July.

It can be seen from Table 11 that the mimosine concentration of tender subabul stems was highest in May (3.90 ± 0.04), lowest in June (3.62 ± 0.03) and medium in July (3.73 ± 0.02). The statistical analysis of data given in Table 12 shows that there were significant differences between the values in May and June ($P<0.01$), May and July ($P<0.01$) and June and July ($P<0.01$).

The average mimosine contents of subabul seeds (Table 11) during May, June and July were 10.70 ± 0.08, 10.44 ± 0.05 and 9.51 ± 0.04 per cent respectively. The statistical analysis of data given in Table 12 shows significant differences between the values obtained in May and June ($P<0.05$), May and July ($P<0.01$) and June and July ($P<0.01$).

It can be seen from the summarised data presented in Table 11 and its statistical analysis in Table 13 that during the month of May, the mimosine content of immature leaves was significantly higher than that of mature leaves ($P<0.01$), tender stem ($P<0.01$) and seeds ($P<0.05$). It can also be seen that while the mimosine concentration in mature leaves was significantly higher than that of tender stem ($P<0.01$), the same was significantly lower than that of seeds ($P<0.01$) during May and that the concentration in the tender stem was significantly lower than that of seeds ($P<0.01$). The data for the mimosine concentrations of immature leaves, mature leaves, tender stem and seeds for the months of June as well as July also showed the same trend of differences between them.

The summarised data presented in Table 11 shows that the mimosine content of immature leaves varied from 9.96±0.05 to 12.11±0.05 per cent, while that of mature leaves, tender stem and seeds varied from 4.89±0.02 to 5.23±0.03; 3.62±0.03 to 3.90±0.04 and 9.51±0.04 to 10.70±0.08 per cent respectively. The high mimosine content of immature leaves (9.96±0.05 to 12.11±0.05 per cent) recorded in the present study appears to be essentially in keeping with the observation of Jones (1979) that mimosine concentration in the growing tips of leucaena may attain a level of 12 per cent, even though the values reported by him for young leaves and seeds were 3 to 5 per cent and 4 to 5 per cent resepectively on dry matter basis. The markedly low mimosine content of 3 to 5 per cent reported by the same author for young leaves compared to the much higher value of 9.96±0.05 to 12.11±0.05 per cent obtained for immature leaves in the present study might be due to the difference in the age of leaves used and/or due to the inherently higher mimosine concentration in the leucaena used in the present study. Hongo _et al_.(1983) also concluded from their studies that the mimosine content was higher for the segment of active growth. According to Jones (1980) fresh _L_.

leucocephala contained 2 to 9 per cent mimosine. Lowry (1981) concluded from his studies that in a typical growing stem the mimosine content can vary from 6 to 1.5 per cent depending on age and position of leaf.

The mimosine contents recorded for different parts of L. leucocephala in the present study are all much higher than those reported by Akbar and Gupta (1984). According to him both immature leaves and seeds of L. leucocephala (Cv.k-8) contained almost similar amounts of 5.06 and 5.04 per cent respectively of mimosine, while mature leaves and branches contained on an average 2.96 and 2.21 per cent respectively of mimosine. It can be seen from Table 11 that in the present study also both immature leaves and seeds contained much higher concentrations of mimosine than those of other edible parts.

In vitro degradation of mimosine

From the data on *in vitro* degradation of mimosine presented in Table 14 to 19 and Fig. 4, it can be seen that the average mimosine concentrations at 0, 12, 24, 36 and 48 hr after incubation of goat rumen liquor with 37.50 mg/100ml of added mimosine were 35.16 ± 0.21, 30.41 ±0.61 , 26.18± 0.52, 24.78± 0.43 and 23.98 ±0.37 mg/100ml respectively with pure mimosine; 35.33 ±0.19,30.35 ±0.46, 26.44±0.23, 24.11±0.28 and 23.14±0.37 mg/100ml respectively with immature leaves; 35.33 ±0.28,29.61 ±0.52, 25.85 ±0.30, 23.41 ±0.34 and 22.20 ±0.28 mg/100 ml respectively with mature leaves; 35.33 ±0.24,29.88 ±0.42, 27.26 ±0.24, 24.64 ±0.56 and 23.12±0.52 mg/100 ml respectively with tender stems and 35.06±0.17, 29.11 ±0.21, 26.12 ±0.84,24.22±0.29 and 23.35 ±0.37 mg/100 ml respectively with seeds. The statistical analysis of data given in Table 20 show that the differences in mimosine concentrations at 0 Vs 12, 0 Vs 24, 0 Vs 36, 0 Vs 48, 12 Vs 24, 12 Vs 36, 12 Vs 48, 24 Vs 36, 24 Vs 48 and 36 Vs 48 hr in respect of pure mimosine (T1), immature leaves (T2), mature leaves (T3), tender stem (T4) and seeds (T5) respectively were all statistically significant ($P < 0.01$ for all except 24 Vs 36 hr in respect of pure mimosine where $P < 0.05$).

It can be seen from the summarised data given in Table 19 and from the statistical analysis of the same given in Table 21 that the 0 hr mimosine concentrations were essentially the same for all the sources of mimosine tested for degradation viz., T1 Vs T2 , T1 Vs T3, T1 Vs T4, T1 Vs T5, T2 Vs T3, T2 Vs T4, T2 Vs T5, T3 Vs T4, T3 Vs T5 and T4 Vs T5. The mimosine concentrations at 12 hr of incubation also were not significantly different for different sources, but for the significantly higher concentration ($P < 0.05$) with immature leaves than that with seeds. At 24 hr of incubation, the mimosine concentration was significantly higher with tender stem than those with immature leaves (P < 0.01), mature leaves (P < 0.01) and seeds (P < 0.05). At 36 hr of incubation the mimiosine concentration with mature leaves was significantly lower than those with pure mimosine (P <0.05), immature leaves (P< 0.05), tender stem (P <0.05) and seeds (P < 0.05) even though there were no significant differences in concentrations in respect of T1 Vs T2, T1 Vs T4, T1 Vs T5, T2 Vs T4, T2 Vs T5 and T4 Vs T 5 . At 48 hr of incubation there were no significant differences between different treatment pairs except that with mature leaves, the mimosine concentration was significantly lower than those with immature leaves (P < 0.01) and pure mimosine (P <0.01) respectively.

It can be seen from the data given in Tables 22 to 27 and Fig. 5 that the percentages of degradation of mimosine at 12, 24, 36 and 48 hr of incubation were 13.48 ±1.72, 25.45 ± 1.70, 29.49 ± 1.27 and 31.69±1.02 respectively with pure mimosine; 14.12 ± 1.07, 25.14 ± 0.96, 31.74 ± 0.90 and 34.49±1.18 respectively with immature leaves; 16.21 ± 1.30, 26.81 ±0.89, 36.43 ± 2.81 and 37.12 ± 0.99 respectively with mature leaves; 15.44±0.88, 22.31 ±0.68, 30.22±1.60 and 34.54±1.50 respectively with tender stems and 16.97±0.53, 25.52 ±0.84, 30.89±0.89 and 33.41±1.03 respectively with seeds. The statistical analysis of data on percentage degradation of mimosine given in Table 28 shows that the difference in the percentages of degradation in respect of 12 Vs 24 hr, 12 Vs 36 hr, 12 Vs 48 hr, 24 Vs 36 hr, 24 Vs 48 hr and 36 Vs 48 hr in regard to T1, T2, T3, T4 and T5, respectively were all statistically significant ($P < 0.01$) for all except those between 24 Vs 36 hr and 36 Vs 48 hr in respect of T1 ($P < 0.05$) and between 36 Vs 48 hr in respect of T3 where there is no significant difference.

It can be seen from statistical analysis of data given in Table 29 that in regard to percentage degradation of mimosine there was no significant difference between T1 Vs T2, T1 Vs T3, T1 Vs T4, T1 Vs T5, T2 Vs T3, T2 Vs T4, T2 Vs T5, T3 Vs T4, T3 Vs T5 and T4 Vs T5 at 12 hr of incubation. At 24 hr of incubation the percentage degradation of mimosine of tender stem was significantly lower than that of immature leaves ($P < 0.05$), mature leaves ($P < 0.01$) and seeds ($P < 0.05$). At 36 hrs of incubation the percentage degradation of mimosine of mature leaves was significantly higher than that of pure mimosine ($P < 0.01$) and tender stem ($P < 0.01$). At 48 hr, the percentage degradation of mimosine of mature leaves was significantly higher than that of pure mimosine ($P < 0.01$) and immature leaves ($P < 0.05$).

From a perusal of the summarised data on mimosine concentration and percentage degradation (Table 19 and 27) and statistical analysis of the same (Table 20, 21, 28 and 29) it can be seen that the average mimosine degradation was maximum with mature leaves and minimum with pure mimosine, the values being intermediate with immature leaves, tender stems and seeds. Tangendjaja et al. (1983) also observed that the degradation of mimosine of dry leucaena leaf during incubation in rumen fluid was faster than that for the pure mimosine. According to him the enzyme present in the dried leaf are rendered active by the absorption of water and the same is more efficient in degrading mimosine than those of the micro organisms in the rumen liquor. Rapid degradation of mimosine to 3-4 dihydroxy pyridine (DHP) by an enzyme present in macerated green leucaena leaves was also reported by Lowry et al. (1983). Tangendjaja et al. (1984) reported that more than 80 per cent of the mimosine was degraded by heating intact leucaena leaves at 70°C for 10 minutes and that the degradation of mimosine can be affected either by the leucaena leaf enzymes or by the enzymes associated with the microflora of the rumen. But according to them, the proteolytic bacteria would inactivate plant enzymes when they gain access to them. In the rumen, therefore, the relative extent of degradation of mimosine caused by microbial and plant enzymes could depend on the accessibility and affinity of the substrate to both sources of enzyme and to the rate at which the rumen microbes degraded plant enzymes. With diets that supported few rumen microbes, mimosine degradation might be largely associated with plant enzymes where as the converse could apply with better quality diets.

While there was appreciable degradation of mimosine from 0 to 48 hr in the present study, Sunil Kumar et al. (1987) observed that there was no degradation of pure mimosine upto 10 hr of incubation with buffalo rumen liquor and that it picked up only after 20 hr of incubation. At 50 hr of incubation, however, the extent of mimosine degradation was 100, 87.2 and 27.7 per cent by rumen fluid, bacteria and protozoa fractions respectively as against highest degradation of 37.12±0.99 per cent recorded with mature leaves and lowest rate of 31.69±1.02 per cent with pure mimosine at 48 hr of incubation in the present study. Rapid degradation of mimosine by rumen fluid from goats (Shiroma and Akashi, 1976), sheep and cattle (Tangendjaja et al., 1983; Kudo et al., 1984) than what is recorded in the present study may be due to the type of the diet and animal species difference. It can be seen from Table 19 that even though mimosine was added to have an initial concentration of 37.50 mg/100 ml in all treatments, the average 0 hr concentrations were only 35.16±0.21, 35.33±0.19, 35.33±0.28, 35.33±0.24, 35.06±0.17 mg/100ml for pure mimosine, immature leaves, mature leaves, tender stems and seeds respectively with an average 0 hr loss of 6.17±0.58, 5.78±0.53, 5.79±0.77, 5.79±0.63 and 6.50±0.48 per cent respectively. Shiroma and Akashi (1976) from their studies on the degradation of mimosine in Leucaena leucocephala de Wit by crossbred (Saanen and Okinawar native breed) goat rumen fluid found a recovery rate of mimosine ranging from 64 to 73 per cent (27 to 36 per cent loss) at 0 hr of incubation. According to them these low recoveries seem to be due partly to loss and partly to its degradation by the micro organisms and enzymes in the medium during manipulation of samples.

The relationship between mimosine concentration and incubation time was more of a quadratic ($Y = a + bx + cx^2$) than of a linear model (Fig.6) as adjudged by R^2 values (Table 30).

It can be seen from Table 31 that the average rate of disappearance of mimosine in $\mu g.\ ml^{-1}.\ h^{-1}$ during the time intervals 0 to 12, 12 to 24, 24 to 36 amd 36 to 48 hr of incubation were 3.95, 3.52, 1.16 and 0.67 respectively for pure mimosine; 4.15, 3.26, 1.94 and 0.81 respectively for immature leaves, 4.77, 3.13, 2.03 and 1.00 respectively for mature leaves, 4.54, 2.18, 2.18 and 1.26 respectively for tender stems and 4.96, 2.49, 1.58 and 0.73 respectively for seeds, the overall average for the entire period of 48 hr being 2.33, 2.54, 2.74, 2.54 and 2.44 respectively for the different sources of mimosine. All the sources of mimosine showed highest rates of disappearance during the first 12 hr of incubation, lower rates during 24 to 36 hr and least rates during 36 to 48 hr of incubation showing a marked reduction in the rate of degradation of mimosine.

Kudo et al. (1984) from their *in vitro* studies using rumen fluid from sheep in Canada found that the relationship between mimosine concentration and incubation time was non linear and that mimosine was degraded more rapidly during the early hrs of incubation, the average rates being 9.00 $\mu g.mL^{-1}.h^{-1}$ for inocula from sheep fed the pelleted diets. Rapid early rates during *in vitro* studies were also observed by Jones and Megarrity (1983) in rumen fluid from goats fed leucaena. According to Kudo et al. (1984) the rapidity of the early rate of metabolism suggest the involvement of fragile microorganisms incapable of sustaining activity. Allison et al. (1983) reported that mimosine degradation was affected by obligately anaerobic gram negative rods and these bacteria appear to be highly susceptible to changes in anaerobiosis. The anaerobic conditions are more favourable during initial stages of incubation and there is less disturbance due to sampling. Alternatively, slower subsequent rates could be due to the limitaions of the *in vitro* system based on strained rumen fluid without additional energy source or buffer.

Effect of mimosine on rumen microbes

Volatile Fatty Acid production

The data presented in Table 32 to 38 and the statistical analysis given in Table 39 show that in regard to *in vitro* VFA producion in m.mol/100ml rumen liquor, there was no significant difference between control (C) Vs T1, C Vs T2, C Vs T3, C Vs T4, C Vs T5, T1 Vs T2, T1 Vs T3, T1 Vs T4, T1 Vs T5, T2 Vs T3. T2 Vs T4, T2 Vs T5, T3 Vs T4, T3 Vs T5 and T4 Vs T5 at 0 hr of incubation. However, at 12, 24 and 36 hr of incubation there were significant differences between C Vs T1 ($P<0.01$), T1 Vs T4 ($P<0.01$) and C Vs T2 ($P<0.05$) respectively, there being no significant difference between other treatment pairs. At 48 hr of incubation, there were significant differences between C Vs T2 ($P<0.01$), T1 Vs T2 ($P<0.05$) and T2 Vs T3 ($P<0.01$) respectively while there were no significant differences between other treatment pairs.

From the statistical analysis of data given in Table 40 it can be seen that the differences in VFA concentrations between all time intervals of incubation in respect of all treatments were statistically significant ($P<0.01/0.05$) except for T3, T4 and T5 in respect of values pertaining to 0 Vs 48 hr of incubation. It can be seen from Table 38 and Fig. 7 that there was an initial increase in VFA concentration, reaching the peak at 12 hr of incubation for all treatments and there after there was a gradual decline upto 48 hr of incubation.

Ammonia production

It can be seen from the data presented in Table 41 to 47 and the statistical analysis given in Table 48 that in regard to ammonia concentration in mg/100ml of rumen liquor there was no statistically significant difference between all treatment pairs at 0 and 12 hr of incubation of rumen liquor. At 24 hr of incubation, however, there were significant differences between C Vs T1 ($P<0.01$), C Vs T2 ($P<0.01$), C Vs T3 ($P<0.01$), C Vs T4 ($P<0.01$), C Vs T5 ($P<0.01$), T1 Vs T4 ($P<0.05$) and T1 Vs T5 ($P<0.01$). While at 36 hr of incubation there was no significant differences between the ammonia concentrations of any of the treatment pairs tested, at 48 hr of incubation there were significant differences between T1 Vs T3 ($P<0.01$), T3 Vs T4 ($P<0.05$) and T4 Vs T5 ($P<0.01$). It can be seen from Table 47 that no ammonia could be detected in control tubes both at 36 and 48 hr of incubation.

The statistical analysis of data on ammonia concentration at various intervals of time given in Table 49 and from Fig. 8 it can be seen that in respect of all treatments there was significant increase in the concentration of ammonia from 0 to 12 hr of incubation ($P<0.01$) and then there was gradual decline in concentration during subsequent periods of incubation, there being significant decrease in concentrations from 0 to 24 ($P<0.01$), 0 to 36 ($P<0.01$), 0 to 48 ($P<0.01$), 12 to 24 ($P<0.01$), 12 to 36 ($P<0.01$), 12 to 48 ($P<0.01$) and 24 to 36 ($P<0.01/0.05$) hr of incubation except that the ammonia concentration remained essentially the same during 24 and 36 hr of incubation in respect of pure mimosine. From 24 to 48 hr of incubation there was significant decrease in ammonia concentration in respect of immature leaves, mature leaves and seeds ($P<0.01$), while the differences between the values at 36 and 48 hr of incubation were significant in respect of mature leaves ($P<0.01$) and tender stem ($P<0.05$).

The production of ammonia and volatile fatty acids (VFA) by rumen micro organisms was taken as an index of their activity in order to find out the effect of mimosine on rumen microbial activity. While Reisner et al. (1979) observed that mimosine is toxic to micro organisms, Sunil Kumar et al. (1987) reported that mimosine did not affect the rumen microbial activity at 2 $mg.ml^{-1}$ of rumen fluid.

According to them the *in vitro* production of ammonia in the presence of mimosine was much higher after 20 hr of incubation than in the control and increasing the concentration of mimosine from 4 to 12 $mg.ml^{-1}$ rumen fluid further increased the production of both ammonia and VFA especially when incubated for 20 hr.

It can be seen from Table 41 to 47 and Fig. 8 that there was an initial increase in ammonia production reaching the peak at 12 hr of incubation and then there was a sudden fall from 12 to 24 hr of incubation. There was, however, no detectable amount of ammonia during subsequent periods of incubation. Similarly, there was an initial increase in VFA production reaching the peak at 12 hr of incubation and there after there was a gradual decline in the concentration. In fact, the production of ammonia and VFA coincided with the active degradation of mimosine, there being faster degradation upto 12 hr of incubation with highest concentration of ammonia and VFA at 12 hr of incubation. The data, thus, showed that rumen microbes degraded mimosine and produced ammonia and VFA. Smith and Fowden (1966) reported the production of DPH, pyruvate and ammonia from mimosine when incubated with the enzyme isolated from 3 day old leucaena seedlings. It is possible that rumen micro organisms also degrade mimosine in the same way. The VFA might have arisen from pyruvate as it is the main substrate for VFA formation by rumen micro organisms (Baldwin and Allison, 1983).

From an overall evaluation of the results obtained during the course of the present study, it can reasonably be concluded that the rumen microflora of Saanen Malabari crossbred goats have only a limited ability to degrade mimosine present in a fairly high concentration in all edible parts of locally available variety of L. leucocephala. It appears that the rumen microorganisms degrade mimosine to form DPH, ammonia and VFA and that mimosine does not affect the rumen microbial activity. The possible role of leucaena endogenous enzymes in the partial degradation of mimosine recorded in the present study cannot however, be ruled out not only in view of the poor rate of disappearance of mimosine but also due to the poor extent of degradation achieved even after 48 hr of incubation with SRL of goat. It is however, quite likely that the rate as well as extent of degradation of mimosine can be improved by proper dietary manipulations which may affect rumen microbial activity.

Summary

SUMMARY

An investigation was carried out to find out the extent of *in vitro* microbial degradation of pure mimosine and that of immature leaves, mature leaves, tender stems and seeds of L. leucocephala using strained rumen liquor obtained from three rumen fistulated Saanen Malabari crossbred goats maintained under standard conditions of feeding and management. The proximate chemical composition and mimosine content of different edible parts of leucaena during the months of May, June and July were determined.

Results of proximate analysis showed that the immature leaves and seeds had significantly higher crude protein content of 30.80 ± 0.19 and 26.22 ± 0.20 per cent respectively than of mature leaves which contained on an average 25.75 ± 0.15 per cent. More of crude fat was stored in seeds (8.30 ± 0.05 %) than in any other edible parts of leucaena. While tender stems contained the highest percentage of crude fibre (35.83 ± 0.25 %), immature leaves had the lowest crude fibre content (9.53 ±0.05 %). The total ash content of leucaena ranged from 4.46 ± 0.08 per cent in seeds to 11.13 ± 0.09 per cent in mature leaves. There was not much variation between different

edible parts of subabul in respect of nitrogen free extract content which ranged from 43.87 ± 0.46 per cent in tender stems to 46.75 ± 0.23 per cent in seeds.

The average mimosine concentrations of immature leaves, mature leaves, tender stems and seeds were 12.11 ± 0.05, 4.89 ± 0.02, 3.90 ± 0.04 and 10.70 ± 0.08 per cent respectively during May; 11.66 ± 0.06, 5.23 ± 0.03, 3.62± 0.03 and 10.44 ± 0.05 per cent respectively during June and 9.96 ± 0.05, 4.92 ± 0.03, 3.73 ± 0.02 and 9.51 ± 0.4 per cent respectively during July on a dry matter basis. The mimosine content of different edible parts of subabul varied significantly between May Vs June, May Vs July and June Vs July except that in respect of mature leaves the values were essentially the same during May and July. There were significant differences between different edible parts in respect of their mimosine content during all the three months studied.

The data on *in vitro* degradation of mimosine revealed that the average mimosine concentrations of strained goat rumen liquor incubated with 37.50 mg /100 ml of added mimosine in pure form or as immature leaves, mature leaves, tender stems and seeds, showed significant reduction due to degradation at every 12 hr intervals from 0 to 48 hr of incubation, the final average respective concentrations being 23.98 ± 0.37, 23.14 ± 0.37, 22.20 ± 0.28, 23.12 ± 0.52, 23.35 ± 0.37 mg /100 ml of SRL. While the average mimosine concentrations at 0 hr of incubation was essentially the same with pure mimosine, immature leaves, mature leaves, tender stems and seeds, at 12 hr of incubation the same was significantly higher with immature leaves than that with seeds. At 24 hr of incubation, the mimosine concentration was significantly higher with tender stem than those with immature leaves, mature leaves and seeds. At 36 hr of incubation the mimosine concentration with mature leaves was significantly lower than those with pure mimosine, immature leaves, tender stems and seeds. The mimosine concentrations were essentially the same for different treatment pairs at 48 hr of incubation except that with mature leaves, it was significantly lower than those with immature leaves and pure mimosine.

The percentages of *in vitro* degradation of pure mimosine and that of different edible parts of subabul by the SRL of goat increased significantly at every 12 hr intervals of incubation from 0 to 48 hr except with mature leaves, which showed no significant increase from 36 to 48 hr. There was, however, incomplete degradation with all mimosine sources, the average percentage degradation at 48 hr of incubation being 31.69 ± 1.02, 34.49 ± 1.18, 37.12 ± 0.99, 34.54 ± 1.50 and 33.41 ± 1.03 with pure mimosine, immature leaves, mature leaves, tender stems and seeds respectively. At 24 hr of incubation the percentage degradation of mimosine of tender stem was significantly lower than that of immature leaves, mature leaves and seeds, even though at 12 hr of incubation there was no significant difference between different sources. The percentage degradation of mimosine of mature leaves was significantly higher than that of pure mimosine and tender stems, at 36 hr of incubation and at 48 hr of incubation, it was higher than that of pure mimosine and immature leaves.

Average 0 hr losses of 6.17 ± 0.58, 5.78 ± 0.53, 5.79 ± 0.77, 5.79 ± 0.63 and 6.50 ± 0.48 per cent respectively recorded during the *in vitro* degradation studies with pure mimosine, immature leaves, mature leaves, tender stems and seeds might be due partly to loss of mimosine and partly to its degradation by the microorganisms and enzymes in the medium during manipulation of samples.

The overall average rate of disappearance of mimosine in $\mu g. ml^{-1}. h^{-1}$ for the entire period of 48 hr incubation were 2.33, 2.54, 2.74, 2.54 and 2.44 with highest rates during the first 12 hr, lower rates during

24 to 36 hr and least rates during 36-48 hr showing a marked reduction in the rate of mimosine disappearance in 48 hr of *in vitro* incubation.

There was an initial increase in VFA concentration, reaching the peak at 12 hr of incubation for all treatments and thereafter there was a gradual decline upto 48 hr of incubation, there being significant differences between C Vs T1, T1 Vs T4 and C Vs T2 at 12, 24 and 36 hr of incubation respectively and between C Vs T2, T1 Vs T2 and T2 Vs T3 at 48 hr of incubation.

There was significant increase in the ammonia concentration from 0 to 12 hr of incubation and then there was gradual decline in concentration during subsequent periods of incubation as in the case of VFA, there being significant difference between C Vs T1, C Vs T2, C Vs T3, C Vs T4, C Vs T5, T1 Vs T4 and T1 Vs T5 at 24 hr of incubation and between T1 Vs T3, T3 Vs T4 and T4 Vs T5 at 48 hr of incubation.

The production of ammonia and volatile fatty acids by rumen microorganisms was taken as an index of their activity in order to assess the effect of mimosine on rumen microbial activity. The production of ammonia and volatile fatty acids coincided with the active degradation of mimosine , there being faster degradation upto 12 hr of incubation with highest concentration of ammoina and VFA at 12 hr of incubation.

The overall results indicated that the rumen microorganisms of crossbred goats degrade mimosine to DPH, ammonia and VFA and that mimosine does not inhibit the microbial activity, even though the possible role of leucaena endogenous enzymes in the partial degradation of mimosine recorded in the present study cannot be ruled out.

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MICROBIAL DEGRADATION OF MIMOSINE IN GOATS

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ABSTRACT OF A THESIS

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ABSTRACT

An investigation was carried out to find out the extent of *in vitro* microbial degradation of pure mimosine (T1) and that of immature leaves (T2), mature leaves (T3), tender stems (T4) and seeds (T5) of L. leucocephala using strained rumen liquor obtained from three rumen fistulated Saanen-Malabari crossbred goats maintained under standard conditions of feeding and management. The proximate chemical composition and mimosine content of different edible parts of leucaena during the months of May, June and July were determined.

While immature leaves and seeds had higher crude protein content,seeds had higher crude fat, tender stems had higher crude fibre and mature leaves had higher ash content compared to other edible parts of subabul.

The average mimosine concentrations of T2, T3, T4 and T5 were 12.11 ± 0.05, 4.89 ± 0.02, 3.90 ± 0.04 and 10.70 ± 0.08 per cent respectively during May; 11.66 ± 0.06, 5.23 ± 0.03, 3.62 ± 0.03 and 10.44 ± 0.05 per cent respectively during June and 9.96 ± 0.05, 4.92 ± 0.03, 3.73 ± 0.02 and 9.51 ± 0.04 per cent respectively during July on a dry matter basis.

The average mimosine concentrations of strained goat rumen liquor incubated with 37.50 mg/100 ml of added mimosine in pure form or as immature leaves, mature leaves, tender stems and seeds showed significant reduction at every 12 hr intervals from 0 to 48 hr of incubation, the final average concentrations being 23.98 $\pm$0.37, 23.14$\pm$0.37, 22.20$\pm$0.28, 23.12$\pm$0.52, 23.35$\pm$0.37 mg/100 ml of SRL.

The percentages of *in vitro* degradation in respect of T1, T2, T3, T4 and T5 increased significantly at every 12 hr intervals of incubation from 0 to 48 hr, even though the degradation was incomplete with all treatments, the average percentage degradation at 48 hr of incubation being 31.69$\pm$1.02, 34.49$\pm$1.18, 37.12$\pm$0.99, 34.54$\pm$1.50 and 33.41$\pm$1.03 respectively.

The overall average rate of disappearance of mimosine in $\mu g.ml^{-1}.h^{-1}$ in respect of T1, T2, T3, T4 and T5 for the entire period of 48 hr of incubation were 2.33, 2.54, 2.74, 2.54 and 2.44 respectively with highest rates during 0 to 12 hr, lower rates during 24 to 36 hr and least rates during 36 to 48 hr.

The production of ammonia and VFA coincided with the active degradation of mimosine, there being faster degradation upto 12 hr of incubation with highest concentrations of ammonia and VFA at 12 hr of incubation.

The overall results indicated that the rumen microorganisms of crossbred goats degrade mimosine to DPH, ammonia and VFA and that mimosine does not inhibit the microbial activity, even though the possible role of leucaena endogenous enzymes in the partial degradation of mimosine recorded in the present study cannot be ruled out.