Mimosine Toxicity - A Problem of *Leucaena* Feeding in Ruminants

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*Leucaena leucocephala* (Lam.) is a vigorous, rapidly growing, drought tolerant, palatable and high yielding tropical or subtropical legume (With annual rainfall of 500 to 3000 mm), enriched in protein (25-35% CP) and other nutritional components.

It is considered a miracle tree for its protein rich foliage (20-30%), fast growing habit, drought tolerance, good high energy fuel, organic nitrogenous fertilizers and its charcoal gum etc. Multi-purpose uses in industries, drought and pest resistance capabilities, effectiveness to control soil erosion and properties to fix nitrogen in soil all made it something legendary. In wet tropics, yield of 20t dry matter/ha/year have been obtained with crude protein yields in excess of 3t/ha.

It was introduced in India in 19th century and was reported to occur in Andamans islands. Extensive studies were carried out on various aspects of *L. leucocephala* at BAF, Urdu Kanchan (Pune); HAU, Hissar; IGRI, Jhansi; IVRI, Izatmagar; PAU, Ludhiana; NDRI, Karnal and several other places. Due to high yield of good quality fodder and other qualities, the *L. leucocephala* was introduced in several states of India and favorable results were reported (Kaul et al., 1983).

In spite of excellent source of nutrients, *L. leucocephala* forage as well as seed contains a numbers of toxic constituents which may severely limit its utilization in livestock. The poor nutritive value of *Leucaena arisae* principally from its toxic amino acid mimosine and its immediate degradation products, 3-hydroxy-4 (1H)-pyridone (Ram et al., 1994). The mimosine content varied in different parts of a plant and also depending on seasons and maturity (Kumar, 2003). In leaf meal and hay it varies from 1.02 to 5.56% of DM. The concentration of mimosine in the growing tips of the leaves may reach up to 12% and in pods to 5% of DM. The mimosine concentration in seeds is within the range of 3.61 to 5.04% of DM (Gampawar et al., 1988).

Tannin concentrations are higher in the leaf meal than in seed. Tannins reduces digestibility of protein and results in marked lower ME value of *Leucaena* leaf meal in poultry. In ruminants, 0.43% condensed tannins (Mahyuddin et al., 1988) present in *Leucaena* Leaf Meal (LLM) may have important nutritional role in the protection of proteins from degradation in the rumen, making it available in the small intestine. Some other metabolically active toxic constituents like protease inhibitor and galactomannan gum are also present in *Leucaena* leaf meal (D’Mello, 1992).

**Chemical Composition of *Leucaena leucocephala***

The Chemical composition of *Leucaena leucocephala* depends on several factors. The major factors include location, variety and age of plant, soil type, season, drying methods. The nutrient composition of *Leucaena* fodder exhibited considerable variations during different months of the year (Singh and Mudgal, 1967). The range of various nutrients are like, DM 24.98 to 36.39, CP 18.9 to 27.57, CF 10.16 to 17.23, EE 2.50 to 5.88, NFE 46.70 to 59.91 and total ash 7.49 to 10.90%. The range of nutrients reported by several other workers were : CP 15.22 to 31.43, CF 7.33 to 16.65, EE 2.50 to 7.10, NFE 33.62 to 57.6, NDF 27.3 to 46.30, ADF 14.4 to 29.79, Cellulose 7.10 to 16.77, Hemicalulose 12.9 to 16.51, Lignin 4.4 to 12.81, total ash 6.8 to 12.5, Ca 0.70 to 2.70, P 0.17 to 0.35%; GE 19.0 to 20.1 MJ kg⁻¹ and β-carotene 227 to 246 mg kg⁻¹ (Upadhya et al., 1974;
D’Mello and Fraser, 1981; Jonas and Megarry, 1983; Makhdoomi and Gupta, 1996; Gupta and Areja, 1999). Garcia et al. (1996) reported that Leucaena leaf meal contained 42% RDP and 48% UDP having amino acid values comparable to that of soybean meal and fish meal with an exception of sulfur amino acids. L. leucocephala pods contained CP 17.50, EE 1.83; CF 27.74, NFE 46.28; total ash 6.65; Ca 0.53 and P 0.32% on DM basis (Radha et al., 1995) and hay contained CP 19.68 to 22.22; CF 9.69 to 16.85; EE 2.88 to 6.60; NFE 48.05 to 51.69; total ash 10.0 to 13.33, Ca 3.63 and P 0.20% of DM (Gupta et al., 1988; Kumar et al., 1987; Yadav et al., 1997). Akbar (1983) reported that DM ranged from 28.33 (immature leaf) to 96.09 (seed), CP 10.32 (stem and dry pod) to 27.62 (seed), EE 3.24 (green pod) to 8.99 (seed), CF 11.03 (seed) to 40.85 (stem), NFE 30.45 (stem) to 48.48 (mature leaves) and total minerals 5.01 (seed) to 12.41 (branch)%. He further reported that Leucaena seeds contained Ca 2.9, P 0.35, S 0.29, Mg 0.33 and K 0.99%; Cu 25 ppm, Mn 875 ppm, Fe 450 ppm and Zn 50 ppm on DM basis. Ramakrishna et al. (1983) studied the effect of cutting height and interval on the composition of L. leucocephala and found that CP increased from 28.30 to 29.77% with increase in cutting height (45 and 115 cm) at both the intervals of 30 and 60 days.

Biodegradation of Mimosine and DHP

Wibaut (1953) has reviewed the chemistry of mimosine which has the structure β-(N-(3-hydroxy-4 pyridine)-amino propionic acid. Hegarty et al. (1964) indicated mimosine can be degraded in rumen. Rumen microflora were postulated to be responsible for degradation of mimosine to 3, 4-dihydroxypyridine (3, 4 DHP)

Conversion was also achieved by rumen microorganisms (Samanta et al., 1998) and caecal microbes in rabbits.

Endogenous enzyme system in the Leucaena plant can also degrade mimosine (Lowry et al., 1983). It was reported that Leucaena seedling have the enzyme which can degrade the mimosine to DHP, pyruvate and NH3. Rumen microflora has the capability of degrading mimosine to 3, 4 DHP as reported by and further 3, 4 DHP to 2, 3 DHP or both to innocuous compound (Ghosh and Areja, 1990b).

Dominguez-Bello and Stewart (1991) found a clostridium strain that degraded mimosine, 3, 4 DHP and 2, 3 DHP to normal metabolites of rumen fermentation in sheep. It was reported 88-89% degradation of mimosine in in vitro rumen studies. Buffalo had the more capability of degradation than cattle (Ram, 1992). Feng and Areja (1998) further reported 74.09% degradation of 3, 4 DHP by cattle rumen liquor in vitro consequent to 3 weeks adaptation to Leucaena leaf meal feeding. The rumen of both cattle and buffalo fed even on conventional diets were found capable of degrading mimosine to 3, 4 DHP and further to 2, 3 DHP. In cattle the 2, 3 DHP degraded to non-toxic compound, whereas in buffaloes through conversion of 3, 4 DHP to 2, 3 DHP was substantial yet further degradation did not occur (Fig. 2 and 3). Susceptible animal could be imparted the ability to degrade 3, 4 DHP and 2, 3 DHP within a week of inoculum transfer either from animal already possessing the ability or with cultures of Synergistes jonsii. The nature of the diet does influence ruminal mimosine metabolism. Kudo et al. (1984) observed that mimosine degradation in vitro was much higher in rumen fluid of animals fed a concentrate diet as compared to those fed a hay diet. Similarly, it was found that mimosine was degraded more rapidly by inocula from sheep receiving oat grain than from those receiving hay alone. However, Feng and Areja (1998) found no effect of diet on mimosine degradation in vitro with rumen fluid collected from both cattle and buffalo.

Influence of Mimosine and its Metabolites in Livestock

Toxicity of mimosine may occur due to inhibition of tyrosine utilizing enzyme or incorporation of mimosine into biologically vital proteins in place of tyrosine (Crouse et al., 1962). Montagna and Yun (1963), in their mice studies revealed gross damage including hair follicle possibly due to inhibition
of mitotic activity by mimosine. However, in another study, it was suggested that mimosine acts on the proliferative phase of growth i.e., mitotic activity in bulbs rather than a keratinization phase (Hegarty et al., 1964). Thyroxine synthesis is hampered due to prevention of the iodination of tyrosine, a metabolic product of mimosine (Hegarty et al., 1976). Peroxidase which is as much necessary for conversion of iodine to iodine radical or nascent iodine which is important for its incorporation into tyrosine is reduced by 3, 4 DHP, therefore, affecting synthesis of T3, T2, T1 and T4. Circulating DHP also may form complex with Zn and Cu (Stanzl et al., 1980) or Fe and lead to excretion of these metals. Mimosine reduced the activity of aspartate amino transferase, polyphenyl oxidase and ATP production was reduced by 70%. DNA synthesis got adversely affected. However, RNA synthesis remained unhindered. Gupta (1995) reported that the 50% level of L. leucocephala leaf meal in rabbit diet reduced the DNA synthesis. Mimosine decreased cell division, DNA, RNA and protein synthesis in Paramecium at submillimolar concentrations.

Effect on Rumen Fermentation

Conflicting results were reported as far as ruminal pH is concerned. There was either no change of pH (Ghosh et al., 2006a) as against a lower (Tumkurirawong et al., 1995) or higher pH values (Mahanta et al., 1998) in ruminal fluid of ruminants fed Leucaena. Further, ruminants fed on Leucaena showed both increased TVFA and acetate concentrations in certain studies (Mahanta et al., 1998) while no significant changes were reported by others (Ghosh et al., 2006a). Molar proportions of propionic acid were found to be decreasing (Gupta et al., 1986) with no changes being observed in butyric acid concentration (Ahmad and Gupta, 1985). The feeding of LLM did not affect protozoal population (Kapoor et al., 1983). However, total-N and NH3-N concentration increased in rumen liquor (Kapoor et al., 1983) or remained unchanged (Mahanta et al., 1998). Goats fed subabul pods also did not reveal any change in pH, TVFA and NH3-N (Radha et al., 1995).

Influence of Leucaena on Milk Yield and Composition

Being cheap and protein rich source Leucaena can produce milk at an economical price, since it is possible to produce 10-22 t of edible DM ha⁻¹ from Leucaena.

Impressive yields of milk in cows have been reported from Leucaena pastures in the tropics, when the dairy cattle were fed approximately one half of dietary dry matter through concentrates. 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 without any toxicity and problem of breeding was also reported. Yet in another study a stocking rate of 4.78 cows ha⁻¹ over a nine months period with 6290 kg milk ha⁻¹ resulted in 272 kg butter fat and 215 kg protein in unsupplemented jersey cows grazing Leucaena pastures in Queensland (Stobbs, 1972). Further a tropical grass diet (18% CP) supplemented with small quantities of the Leucaena showed milk yield (Flores et al., 1979).

No detrimental effect of the amino-acid, mimosine on animal health or performance is observed when Leucaena constitutes only a small proportion of the cows’ diet (Hamilton et al., 1968). Under grazing systems the most efficient method of utilization might be to allow cows for a short period each day to graze Leucaena followed by grazing nitrogen fertilized grass pastures (Jordan et al., 1995).

Rejection of such tainted milk may not be 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., 1968).

Iso-calorie diets with up to 50% of the concentrate-N may be replaced by the tropical tree legumes Gliricidia and Leucaena without reduction in milk production. Leucaena served as an economical substitute for wet breeder’s grain without showing a change in milk yield and its composition (Morillo and Faria, 1996). Further, Leucaena leaf meal replaced 30% of costly groundnut and sunflower cakes without affecting milk production or its composition, thereby reducing the cost of
milk production (Garg and Kumar, 1994). Even inclusion of 15% subabal seeds in concentrate mixture sustained milk production without affecting milk composition, animal health and reproduction (Talpada et al., 1994). Higher milk production as well as increased total solids, Solids-Not-Fat and protein with lower feed cost have also been reported in goats (Rai et al., 1994). Gupta (1995) did not observed any adverse effect on milk yield in goat fed gradually increasing levels of dietary Leucaena DM (up to 75%) but on sole Leucaena feed, a reduction in milk yield was reported and the reduction was attributed to either toxic effects or low nutrient intake. These workers further observed that neither mimosine nor DHP was secreted in goat’s milk, thus rendering it safe for consumption.

Toxicity of Mimosine and Dihydroxy-pyridone in Ruminants

The toxicity of mimosine and its metabolites may vary in different classes of ruminants and in the different geographical regions of the same class of ruminants. The toxicological effects of Leucaena feeding in different classes of ruminants are discussed in the following text.

Cattle

Alopecia, loss of appetite, excessive salivation, in coordination of gait, enlarged thyroid gland and poor breeding performance were reported in cattle grazing on Leucaena. Leucaena fed cows par triturated calves with lower body weight and had nervous symptoms with alopecia during early mid lactation (Hamilton et al., 1968). Enlargement of pituitary gland, neonatal death, low conception rate, poor growth reduced appetite and alopecia and other associated breathing problems were reported in cows (Holmes et al., 1981). The hypertrophy of thyroid gland was observed by Jones and Jones (1982) with no effect on productivity. Steers fed on 67 and 100% Leucaena diets showed the symptoms of low feed intake, loss of body weight and hypothyroidism (Fig. 3). Loss of body weight, increased plasma profile of hepatic enzymes and depression of T3 and T4 levels in plasma were recorded on Leucaena feeding in cattle (Ghosh et al., 2006b). Pachauri and Pathak (1989) reported tongue ulceration and congested buccal cavity in crossbred calves fed on Leucaena forage. Symptoms of emaciation, alopecia, scaly skin, ear and eye lesion, ulceration on mouth region, drooling of viscid saliva and even vomiting of thick green slime were observed in calves (Ram et al., 1994).

Buffalo

Letts (1963) observed alopecia, loss of appetite, excessive salivation, in coordination of movement and enlarged thyroid gland in buffalo calves. Male buffalo calves fed 50% Leucaena reported zero sperm motility in 2 of the 4 buffalo bulls who produced semen (Lohan et al., 1988) and body weight gain was reduced from 590 to 345 g/day. Reduced DM intake and weight gain; alopecia, in coordination of gait; lower levels of T3 and T4 and increased AST and ALT activities in plasma were observed by in buffalo calves (Ghosh, 1998; Gupta, 1995).

Sheep

Poor wool growth and hemorrhagic cystitis were observed in sheep fed on Leucaena diet (Fig. 3). A daily mimosine intake of 0.2-0.3 g/day body weight caused wool shedding in sheep. Decreased body weight, goiter, neonatal death, oesophageal ulcers were reported with defecating in pregnant ewes. Gupta (1995) observed excessive salivation with loss of body weight in sheep fed on Leucaena hay for a period of 17 days. Alopecia, loss of appetite, salivation, loss of weight, low hemoglobin level, PCV, TLC, were observed in sheep fed Leucaena diet (Prasad, 1988). Nephritis and cirrhosis were revealed by Prasad and Pulwai (1989) along with tongue ulceration and thyroid hypertrophy. Shedding of wool and negative phosphorous balance were observed in rams fed on diets containing water treated L. leucocephala. Makhdoumi and Gupta (1996) reported shedding of wool in sheep on sole Leucaena feeding but with 50% LLM in ration no ill effects were found. Loss of body weight on 100% Leucaena feeding has been reported recently (Makhdoumi and Gupta, 1997).

Note: Based on 1st (A), 2nd (B), 3rd (C) and 4th (D) week post LLM feeding observations
Fig. 1: Model: Mimosine kinetics in Leucaena Adapted milch cow

Note: Based on 1st (A), 2nd (B), 3rd (C) and 4th (D) week post LLM feeding observations
Fig. 2: Model: Mimosine kinetics in Leucaena adapted buffalo

Goats

Leucaena feeding caused no toxicity symptoms in goats (Paul et al., 1998). However, alopecia was observed in goats on Leucaena diet (Chakraborty and Ghosh, 1988). Hypothyroidism in goats was also reported in Australia on sole Leucaena feeding (Jones and Megarry, 1983). External supplementation of thyroxine had no effect to reverse the mimosine toxicity (Megarry and Jones, 1983). Black Bengal goats fed ad lib Leucaena forage did not show any clinical symptoms of toxicity
Fig. 3: Model: Mimosine kinetics in *Leucaena* adapted cattle

(Chakraborty and Ghosh, 1988) whereas in studies on alpine X beetal goats fed 89% of CP through *Leucaena* seed, only one goat (Out of eight) showed icterus. Goats fed on *Leucaena* diet exhibited toxicity symptoms after 7 months of feeding (Samenya, 1990). Milk goats having detectable levels of DHP in their blood did not show presence of either mimosine or its metabolites in milk thereby making it safe for human consumption (Fig. 1). Observed mild to severe changes in thyroid gland, hepatic parenchyma and mucosa of intestine after two years of uninterrupted feeding (Level 25-50%). However, 75-100% feeding level decreased the spermatogenic cells in seminiferous tubules, degenerative changes in kidney, myofibrils and hyperplasia of spleen and lymphnode in goats.

**Mimosine Inactivation**

Mimosine and its metabolites are the main hindrance blocks for the utilization of *L. leucocephala* as animal feed. Therefore, the research workers had tried to develop different methods for the elimination of the toxicological effects of mimosine and its metabolites, through physico-chemical or biological techniques as described in the following text.

**Physico-chemical Inactivation**

The treatment of supplementation of LLM with some mineral salts leads to reduction in toxic effects of mimosine. In this context efficacy of iron (Gupta and Atreja, 1997), aluminium (D’Mello and Acadovic, 1982), copper (Ram et al., 1994), calcium in toxicity alleviation due to the chelation effect and thereby excretion through faeces were reported. However, the ferric form was more efficient than ferrous as shown in some studies when used as sulphate or chloride (D’Mello and Acadovic, 1982). Leaching of LLM with 0.05% Sodium acetate detoxified 95% mimosine without loss of any important nutrients (Tawata et al., 1986). L-phenylalanine and L-tyrosine supplementation could partially or completely reduce toxicity of mimosine in rats on the basis of their structural similarities with mimosine. Lowry (1983) revealed that maceration activated the enzyme capable of converting mimosine to DHP. Moist heat at 70 to 100°C was effective to reduce 50% mimosine content (Akbar and Gupta, 1985). Further increase of 40 to 100°C temperatures reduced the mimosine

<table>
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<tr>
<th>Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>Mimosine (g)</td>
<td>15.01</td>
<td>30.03</td>
<td>21.19</td>
<td>16.22</td>
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<tr>
<td>B. Wt. (kg)</td>
<td>169.00</td>
<td>162.00</td>
<td>157.33</td>
<td>139.00</td>
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<td>Mimosine (%)</td>
<td>1.12</td>
<td>0.73</td>
<td>0.26</td>
<td>0.15</td>
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<td>3, 4 DHP (g) (%)</td>
<td>(7.46)</td>
<td>(2.45)</td>
<td>(1.13)</td>
<td>(0.96)</td>
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<tr>
<td>Total (g) (%)</td>
<td>2.7</td>
<td>3.09</td>
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<td>0.39</td>
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<tr>
<td>(18.01)</td>
<td>(10.30)</td>
<td>(3.68)</td>
<td>(2.44)</td>
<td>(1.94)</td>
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<tr>
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<td>0.54</td>
<td>0.72</td>
<td>0.42</td>
<td>1.71</td>
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<td>(0.31)</td>
<td>(1.83)</td>
<td>(1.29)</td>
<td>(2.61)</td>
<td>(11.37)</td>
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<tr>
<td>3.87</td>
<td>4.36</td>
<td>1.31</td>
<td>0.96</td>
<td>2.03</td>
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<tr>
<td>(25.78)</td>
<td>(14.58)</td>
<td>(6.20)</td>
<td>(6.01)</td>
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content by 9.45 to 14.52%. Dry heating at 100°C reduced 17 to 19% mimosine content whereas autoclaving reduced 19 to 23% (Mali et al., 1990). However, there was no beneficial effect of dry heat temperature of Leucaena (Atreja et al., 1990). Other suggested detoxification procedures are ensiling (Tawata et al., 1986) and soaking, cooking and washing (Padmavathy and Shodha, 1987). Molasses supplementation was also documented to reduce the mimosine toxicity (Elliot et al., 1985). Heat treatment (Tangendikjaja et al., 1990) and supplementation of amino acids or metal ions such as Fe²⁺, Al²⁺ and Zn²⁺ (Kumar, 2003) also have toxicity reducing effects for Leucaena and mimosine.

**Biological Inactivation**

In some geographical regions Leucaena feeding did not show any toxicity symptoms in ruminant animals. Hawaiian goats fed Leucaena diets did not show any adverse effects. Jones and Megarrity (1983) observed 71% of adzed DHP was degraded by Hawaiian goat rumen liquor as against no degradation by Australian goat rumen liquor. Therefore, they predicted a different metabolism of mimosine in Hawaiian goats which exhibited no toxicity symptoms in Australian goats. Trans-inoculation of Hawaiian goat rumen liquor to Australian steer fed 100% Leucaena diet revealed no urinary excretion of DHP in the steers (Jones and Megarrity, 1986). They also reported that only bacteria were involved for DHP degradation in the rumen.

Quirk et al. (1988) reported prior introduction of 3, 4 DHP degrading bacteria to the cattle improved weight gain on only Leucaena pastures. The bacteria of rumen fluid from animals in Iowa were incapable of the DHP degradation. However, the animals in Virgin Islands and Haiti had documented a complete reversed picture.

A gram positive spore forming bacterium named as costridium strain 162 degraded 3, 4 DHP and 2, 3 DHP to normal rumen metabolites (Domenguez-Bello and Stewart, 1991). In India 3 to 4 weeks of gradual adaptation caused cattle to acquire the DHP degrading ability (Puniya et al., 1996). This DHP degrading ability could be transferred from cattle to cattle within 9 days. Hammon (1995) showed that susceptible animals could be impacted the ability to degrade 3, 4 DHP and 2, 3 DHP within a week of inoculum transfer either from the animals already possessing this ability or with cultures having active population of Synergistes jossii bacteria. Similarly, Indian workers showed that otherwise susceptible crossbred cattle (Ghosh and Atreja, 1999c) and Murrah buffalo (Ghosh and Atreja, 1999d) could be imparted the ability to degrade 3, 4 DHP and 2, 3 DHP within 5 days of inoculum transfer either from the animal already possessing this ability or with cultures having active population of Streptococcus bovis (Koshy, 1996; Ghosh, 1998). Chhabra et al. (1997) isolated DHP degrading bacteria from rumen fluid of goat fed on Leucaena which could degrade 80 to 90% of both 3, 4 DHP and 2, 3 DHP.

**REFERENCES**


