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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 ware carried out on various aspects of *L. leucocephala* at BAIF, Uruli Kanchan (Pune); HAU, Hissar; IGFRI, Jhansi; IVRI, Izatnagar; PAU, Ludhana; 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 arises* 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 3 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 low 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 2.59 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 38.62 to 57.6, NDF 27.3 to 46.30, ADF 14.4 to 29.79, Cellulose 7.10 to 16.77, Hemicellulose 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} (Upadhyaya *et al*., 1974;

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D'Mello and Fraser, 1981; Jones and Megarrity, 1983: Makhdoomi and Gupta, 1996; Gupta and Atreja, 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.*, 1983; 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. Rangnekar *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 pyridone)-amino proprionic acid. Hegarty *et al.* (1964) indicated mimosine can be degraded in rumen. Rumen microflara 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 Atreja, 1999b).

Domenguez-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 Atreja (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 Atreja (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 (Crounse *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 T_1, T_2, T_3 and T_4. Circulating DHP also may form complex with Zn and Cu (Stunzi *et al*., 1980) or Fe and lead to excretion of these metals. Mimosine reduced the activity of aspertate amino transferase, polyphenyl oxidase and ATP production was reduced by 70%. DNA synthesis got adversely affected. However, RNA synthesis remained unhampered. 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 (Tumkiratiwong *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 (Akbar and Gupta, 1985). The feeding of LLM did not affect protozoal population (Kapoor *et al*., 1983). However, total-N and NH_3 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 NH_3-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 an 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 brewer'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% subabul 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

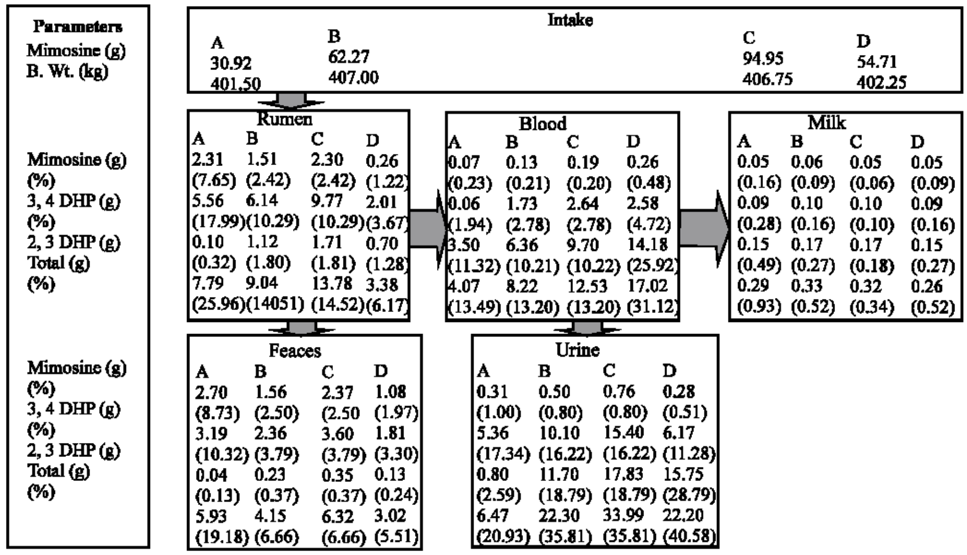
Alopecia, loss of appetite, excessive salivation, in coordination of gait, enlarged thyroid gland and poor breeding performance were reported in cattle grazing on *Leucaena*. *Leucaena* fed cows parturated calves with lower body weight and had nervous symptoms with alopecia during early mid lactation (Hamilton *et al*., 1968). Enlargement of pituitary gland, neonatal death, low conception rate, poor growth reduced appetite alopecia and other associated breeding 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 T_3 and T_4 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 T_3 and T_4 and increased AST and ALT activities in plasma were observed by in buffalo calves (Ghosh, 1998; Gupta, 1995).

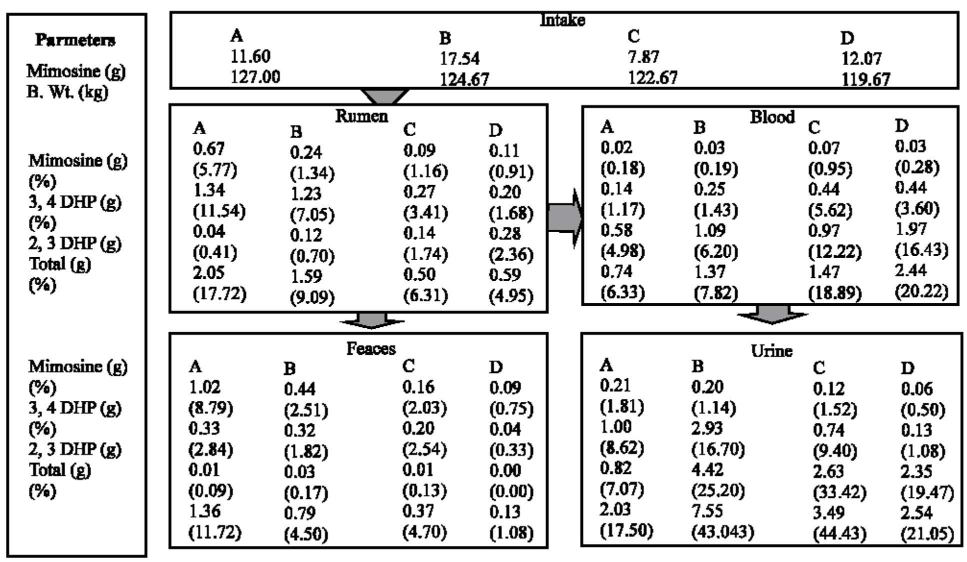
Sheep

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, oeosophageal ulcers were reported with defleecing in pregnant ewes. Gupta (1995) observed excessive salivation with loss of body weight in sheep fed on *Leucaena* hay for a period of 17 day. 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 Paliwal (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*. Makhdoomi 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 (Makhdoomi 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 Megarrity, 1983). External supplementation of thyroxine had no effect to reverse the mimosine toxicity (Megarrity and Jones, 1983). Black Bengal goats fed *ad lib Leucaena* forage did not show any clinical symptoms of toxicity

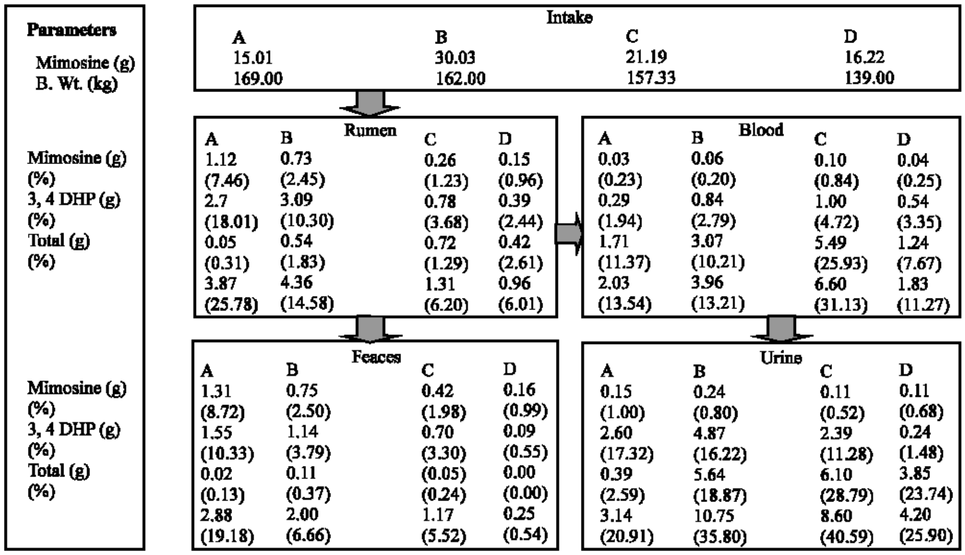


Fig. 3: Model: Mimosine kinetics in *Leucaena* adapted cattle

(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 alopecia. Goats fed on *Leucaena* diet exhibited toxicity symptoms after 7 months of feeding (Semenye, 1990). Milch 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 physio-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 Acamovic, 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 Acamovic, 1982). Leaching of LLM with 0.05N 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 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 (Tangendijaja *et al*., 1990) and supplementation of amino acids or metal ions such as Fe^{2+}, Al^{3+} and Zn^{2+} (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 added 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 clostridium 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. Hammond (1995) showed that susceptible animals could be imparted 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 jonsii* 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.

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