1 Introduction

Ruminants have an important economic role in village systems to provide draft power, meat, manure, hides and milk in most countries of South-East-Asia. It is also evident that high human population densities will continue to ensure that the first priority of land use must be on production of food and plantation crops. In global consideration, this demographic pressure leads to a greater emphasis on the development of livestock and agricultural systems.

The animals, especially monogastric animals, often compete with human beings for food resources. However, ruminants therefore play an important role in this aspect because of their ability to convert feed that cannot be used by monogastric animals into meat and milk for human consumption. It is well recognized that rice straw is the main feedstuff for ruminants. Despite its wide spread use and availability, straw has severe nutritional limitations for ruminants in particular, because of its low digestibility, low crude protein content, high silica, lignin and low intakes. Therefore, ruminants cannot maintain their liveweight when rice straw is fed as sole diet. Supplementation with protein rich feedstuff is one of the common ways to overcome the low quality nutritive value of rice straw, however still being the main limiting factor in ruminants in tropical countries. Therefore, supplementation with fodder leaves is often the only source of nitrogen for ruminants during dry season. Leguminous forages may provide specific substrates and nutrients, such as protein. Cell walls are better fermentable than those of straw and essential minerals and vitamins, which increase microbial activity in the rumen.

Although *Leucaena leucocephala* (leucaena) is one of the recommended forages, it is not a fully satisfied feed for ruminants. It contains a toxic amino acid, mimosine that has antimitotic and depilatory effects on animals. Concentrations in young leaves can be as high as 12 % and the edible fraction commonly contains 4-6 % mimosine. Mimosine is acutely toxic to animals but is normally converted to 3-hydroxy-4(1h)-pyridone (DHP) upon ingestion. DHP is goitrogenic and, if not degraded, can result in low serum thyroxine levels, poor appetite, ulceration of oesophagus and reticulo-rumen, excessive salivation, poor appetite, raw coronet above the hooves, lameness, low liveweight gains, foetal death and resorption especially when the diet contains more than 30 % Leucaena in ruminants. Even it can be controlled by supplementing with minerals; it is very expensive for supplimenting ruminants. The anaerobic rumen bacteria, *Synergestes jonesii* was found in the rumen of some countries in the Americas and South-East Asia. This can completely detoxify DHP and its breakdown products. In the mid 1980s, *S. jonesii* was transferred to ruminants in Australia and subsequently to Africa and China. However, the use of these bacteria has still limitations for end users. This is a strict anaerobic bacterium and takes long time to grow on media plates. On the other hand, it still has difficulties for the end users for storage and inoculation in the ruminants. So, the alternative microbe and/ or ways for storage and inoculation are still needed. Thus, this study was intended:

- To select and isolate mimosine degrading bacteria from rumen juice of German steers,
- To multiply mimosine degrading bacteria by using IBT-Goettinger Bioreactor technology which is suitable for bacterial vaccine production,
- To search common ways of storage and inoculation of microbes for end users,
- To compare *in vivo* ability of isolated mimosine degrading ruminal bacteria (IBT-Göttinger Bioreactor product) with that of *S. jonesii* in Myanmar sheep by using digestion trials,
- To control mimosine toxicosis in ruminants.

2 Literature Review

2.1 Mimosine

Mimosine is an alkaloid, β-3-hydroxy-4 pyridone amino acid. It is a toxic free amino acid. Renz (1936) isolated mimosine from *Mimosa pudica* and named it mimosine. Later, Bickel and Wibaut (1946) also isolated it from *Mimosa pudica*. The chemical structure of mimosine was determined by Bickel *et al.* (1947a, 1947b). It's chemical structure is similar to that of the amino acid tyrosine (Fig. 1). In ruminants, mimosine is degraded to 3,4 and 2,3 dihydroxy pyridone. It occurs in a few mimosa and all species of the closed allied genus *Leucaena* (Kumar, 1999).

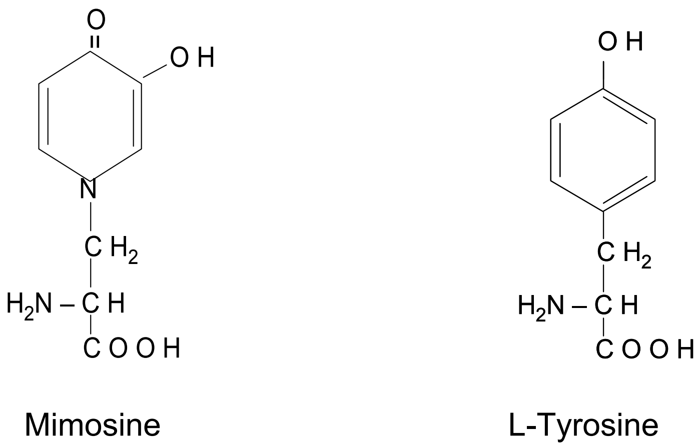

Fig .1 Chemical structures of mimosine and L- tyrosine

2.1.1 Mimosine in Leucaena

The concentration of mimosine in the leaves and seeds of *L. leucocephala* (leucaena) varies with the different types and strains of the plant. Ter Meulen *et al.* (1979) found that mimosine concentration in samples of plants grown in Zaire were 7.19 % and 12.13 % of the total protein in the leaves and seeds. Particularly, high concentrations were found in the tips of actively growing shoots (8-12%), young leaves (4-5%), and young seed and pods (4-5%) (Jones, 1979). Soedarjo and Borthakur (1996) analyzed mimosine content of leucaena. They found that it was 9.06%, 7.83%, and 6.58 % respectively in pods, leaves and seeds. It was described that leucaena contained about 3 to 5 % of mimosine on dry matter basis (Hammond 1994). Akbar and Gupta (1984) found that leucaena seeds contained more mimosine (5.04 % DM) than mature leaves (2.96 % DM), but lower than that in the immature leaves (5.06 % DM). Kale (1987) reported that mimosine contributed as much as 14.8 % to the nitrogen content of leucaena. In other findings, the amount of mimosine in leucaena varied from 2.2 to 10 % (Hongo *et al.,* 1987; Akbar *et al.,* 1991).

Mimosine content of dry matter of the edible parts of leucaena in western Nigeria ranged from 12.3 % in the yellow cotyledon to 0.5 % in the empty green pod. Mature seeds are twice as rich in mimosine as young seeds, 6.15 % and 3.2 %, respectively (Adeneye, 1991). It was similar to the reports from ter Meulen *et al.* (1979), Kale (1987), and Hongo *et al.* (1987). Ter Meulen and El-Harith (1985) reported that concentration of mimosine in seed was higher than that in leaves (3.34 % and 2.13 % respectively). Young and mature leaflets are richer in mimosine than the corresponding petioles. The green and brown seed coats and empty brown pods contain no mimosine (Adeneye, 1991).

2.2 *Leucaena leucocephala* (leucaena)

Species **:** *Leucaena* (Lam.) de Wit
Family **:** *Leguminosae*
Subfamily **:** *Mimosideae*

Fig. 2: Leucaena trees in Myanmar

Fig. 3**:** Leucaena seeds and pods

2.2.1 Description

Much of the confusion over naming *Leucaena leucocephala* results from the fact that its many varieties differ enormously in size and form (Ruskin, 1977). Leucaena has its origins in Central America and Mexico where it has been used by humans for several thousand years and continues to be cultivated by recent day farmers (Hughes, 1993). The genus of *Leucaena leucocephala* was reported to contain 16 (Brewbaker and Sorensson 1993) or 17 species (Hughes, 1993). The most widely planted species is *Leucaena leucocephala* (Lam.) de Wit, know as leucaena and as its fodder values was well recognized, Spanish conquistadors carried leucaena on their galleons to the Philippines to feed their stock and from there, leucaena has been spread to most tropical countries of the world (Brewbaker *et al.,*1985).

Leucaena is a thornless long-lived shrub or tree, which may grow to heights of 7- 20 m. Leaves are bipinnate with 6-8 pairs of pinnae bearing 11-23 pairs of leaflets 8-16 mm long. The inflorescence is of cream coloured globular shape, which produces a cluster of lat brown pods 13-18 mm long containing 15-30 seeds. Three morphological types are noted by Domergues *et al.* (1999), that bushy Hawaiian type is bushy, small and less than five metres; a tall Peruvian type is with several stems to 15 meters; a Hawaiian giant type is with a trunk and great size to 20 metres. The commonest form is the shrubby free-seeding one, which tends to be weedy and low yielding and it was transported around the world from the 16th to 19th centuries and was pan-tropical (Jones, 1979).

2.2.2 Some common names of *Leucaena leucocephala*

Table 1: Some common names of *Leucaena leucocephala* (Brewbaker *et al.,* 1985; Mehm Ko Ko Gyi, 2002)

Common Name	**Countries**
Leucaena	Australia, United States
Ipil Ipil	Philippines
Lamatoro	Indonesia
Katin	Thailand
Yin ho huan	China
Kababul or subabul	India
Koa haole	Hawaii
Tangantangan	Some Pacific Islands
Cassis	Vanuatu
Guaje	Mexico
Huaxin	Central America (Maya)
Bawzagaing	Myanmar

2.2.3 Environmental adaptation

Leucaena is a free-seeding, colonising plant, which has spread to a very wide range of sites, which are more or less frost-free, and has naturalized itself in many areas, some far outside the tropics. In East Africa, it will grow up to about 1,900 meters above sea level with slow growth as altitude increases. It is found as far north as the Punjab and the Himalayan foothills and occasionally in North Africa. It was also seen in many semiarid areas provided that, it could find some soil moisture. To be grown as forage, however, it needs a long warm, moist growing season.

Leucaena requires warm temperatures (25-30°C day temperatures) for optimum growth. At higher latitudes and at elevated tropical latitudes, growth is reduced. Brewbaker *et al.* (1985) suggest that temperature limitations occur: above 1000 m elevation within 10° latitude of the equator, and above 500 m elevation within the 10-25° latitude zones.

Leucaena is not tolerant of even light frosts, which cause leaves to be shed (Isarasenee *et al.,* 1984). Heavy frosts will kill all aerial growth, although the crowns survive and regrow vigorously in the following summer with multiple branches. Shading reduces growth although it has moderate tolerance of reduced light when compared with other tree legumes (Benjamin *et al.,* 1991). Seeds will germinate and establish satisfactorily under established leucaena hedgerows or other tree cover. It has been successfully grown under coconuts in Bali as a support for vanilla.

Leucaena can be found performing well in a wide range of rainfall environments from 650 to 3,000 mm. However, yields are low in dry environments and are believed to increase linearly from 800 to 1,500 mm, other factors being equal (Brewbaker *et al.,* 1985).

2.3 Nutritive value of Leucaena

2.3.1 Leaves

The nutritive value of the feedstuff depends upon its nutrient composition, digestibility, and the appropriate amount of feed intake. Leucaena foliage contains both nutrients and roughage, and makes an almost complete ruminant feed. The N-corrected metabolizable energy (ME) for poultry was 83 ± 0.74 MJ/DM (D'Mello and Thomas, 1978). This value was similar to the ME of the sun dried Alfalfa reported by Scott *et al.* (1969). It was discussed, that the low

ME content was from the low digestibility of this legume (D'Mello and Thomas, 1978). Gieseke (1984) stated that leucaena leaves contained 4.39 ± 0.8 MJ / kg DM and seeds 4.19 ± 1.8 MJ/kg DM.

Leucaena leaves are a high protein feed source because they can contain from 29.2 % to 34 % of crude protein in dry matter respectively (ter Meulen *et al.,* 1979; Kale, 1979). This amount confirmed by Akbar and Gupta (1985), Yadav and Yadav (1988) and Hilal *et al.* (1991). In the experiment of Gieseke (1984), leucaena leaves contained 91.5 % OM, 23.1 % CP, 3.5 % EE, 9.4 % CF and 55.6 % NFE. Aletor and Omdara (1994) studied some leguminous browse plants, with particular reference to their proximate mineral and some indigenous anti-nutritional constituents. They discovered that leucaena leaves contained 25.27 % CP, 18.28 % CF, 5.89 % EE 7.64 % ash and 42.92 % NFE. In their experiment, CP content of leucaena was higher than other leguminous plants. Soedarjo and Borthakur (1996) reported that Leucaena leaves contained 18.77 ± 1.51 % CP in dry matter DM before water soaking and 17.98 ± 0.92 % CP after soaking. The CP content of leucaena leaves in Myanmar is almost the same to the reports from other countries (Ni Ni Maw *et al.,* 2002). They evaluated the nutritive values of 30 types of tree foliages available in Yezin area. They found that leucaena contained 91.22 % OM, 22.00 % CP, 22.92 %NDF, and 16.56 % ADF. However, there is no research work concerned with leucaena toxicity in Myanmar. Khamasekhiew *et al.* (2001) reported that the crude protein content of the edible material (small stem and leaves) of leucaena ranged from 14-30 % and it was accepted that leucaena could constitute up to 30 % of the ration of ruminants without any toxicity.

Not only the protein content of leucaena leaves can make a high nutritional quality, but also amino acids are present in well-balanced proportions of leucaena and are almost the same as in Alfalfa (Hagerty, 1977; Jones, 1979). Ter Meulen *et al.* (1979) reviewed the nutritive value of leucaena. They compared the amino acid values of leucaena leaves and seeds with those of soybean meal and alfalfa. It was reported that glutamic acid, aspartic acid, leucine, and isoleucine are the major components of leucaena (Hilal, *et al.,* 1991).