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Mimosine Toxicity in Leucaena Biomass: A Hurdle Impeding Maximum use for Bioproducts and Bioenergy

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Abstract

Leucaena biomass could serve as a new commodity for bio energy production. It is well established as a feedstock for animal husbandry, due to its nutrient content and lower cost. However, the existence of mimosine caused loss of hair among ruminants. This paper provides a short review of current uses of Leucaena, from agriculture to bio energy. In the next section, the techniques to remove Leucaena toxicity caused by the toxic non-protein amino acid mimosine will be discussed. A common mimosine inactivation technique adopted for animal rations is dosage-dependent on the Leucaena portion and inclusion of iodine, iron, copper or sulphate in the diet. However, this technique is inefficient. Other approaches such as enzymes from ruminal microbes or soil bacteria and low-mimosine Leucaena clones have become new strategies to overcome this problem, although the toxicity is reduced it still can be seen. Future research is needed to if possible fully eliminate the toxicity and realize the goal of Leucaena utilization to produce both bio products and bio energy.

Keywords: Mimosine; Toxicity; Bio Energy; *Leucaena Leucocephala* and Biomass

Introduction

Leucaena leucocephala is a leguminous fast growing tree native to Mexico and Central America, but now found in most of the sub tropic and tropical regions around the world. L. leucocephala belongs to the *Fabaceae* family, with about 22 species in the Leucaena genus worldwide including two named acid-tolerant hybrids (*L. leucocephala Bahru and L. leucocephala Rendang*) [1]. It is known as *Subabul* in India, Ipil-ipil in the Philippines, Yin hue in China and *Petai Belalang* in Malaysia. In this review, *L. leucocephala* will be referred to using its general name, Leucaena. Due to factors such as increasing market demand for beef products, escalating land prices for agricultural activities and high cost of protein concentrates, there is urgency to find alternative animal feed stocks. Feedstock shortages faced by developing countries has driven adoption of Leucaena for its nutritive value, low cost and sustainability. This tree has been extensively introduced as an agro-forestry product and forage legume for its high crude protein, also for being highly palatable, long-lived, and drought tolerant. The improvement of crop yield when intercropping Leucaena with food crops has been widely documented. A study by Imogie reported a noticeable increase in fresh fruit bunch production when intercropping Leucaena with oil palm [2]. Its deep root system and ability to fix nitrogen could aid in soil erosion, soil fertility and aeration, creating a healthy nitrogen cycle in crops. It also has use in bioremediation to treat industrial waste [3]. Numerous papers show cattle fed with Leucaena show a gain in live weight more than with other forage legumes, from 50 kg/ha/yr to 200 kg/ha/yr [4]. Similarly, it is observed that Leucaena intake as a supplement significantly improves the rumen microbial population, improves N-retention and microbial supply, thus contributing to an efficient digestion process. However, inclusion of Leucaena in the diet has been shown to lower the weight of catfish and Nile tilapia when exceeding 20% and 50% of the diet, respectively [5,6].

Current Uses

Table 1: Biomass harvest from Leucaena by different methods.

Biofuel Types	Methods	References
Biomass based power generation	Gasification of Leucaena's wood	[13]
Bio ethanol	Fermentation of Leucaena's legumes	[14]
Biodiesel	Microwave assisted irradiation of Leucaena's legumes	[14]
Bio-oil	Pyrolysis of Leucaena's trunk	[15]
Bio char	Pyrolysis of Leucaena's bark	[16]

Unlike common ruminant diets that contribute to methane (CH4) emission, papers have shown that Leucaena pastures were able to mitigate the greenhouse gas emissions by approximately 91,000 t carbon dioxide equivalent carbon (CO_{2-e}) annually [7]. This anti-methanogenic characteristic is due to the presence of a plant secondary compound known as condensed tannins (CT). CT has protein binding properties and could form CT-protein complexes that prevent the protein from being degraded into CH4 in the rumen, thus enabling the protein to escape to the ruminant intestine [8]. The dynamic uses of Leucaena have shifted the world's interest to explore its potential as a fuel crop. The composition of Leucaena provides incentive for industries to utilize it, particularly in wood, pulp and paper production. Studies showed that Leucaena liquor obtained by auto hydrolysis gives a potential energy yield. In wood production, Leucaena has met the requirement set by the EN standard for general purpose wood, with a target density of 700 kg/m^3, making it a suitable candidate raw material in wood composite manufacture [9,10]. It was suggested that the rapid growth and high dry matter production of Leucaena serves as a potential biomass for generating electricity, based on the heating value and wood density as it requires about 1.5kg of dry wood to produce 1kwhr^{-1} [11]. Likewise, it was found that Leucaena feasibility for bio ethanol in the motor gasoline industry and its by-product electricity generation could meet 8% of state wide energy demand [12]. It is notable that factors such as plant spacing has significant effect on plant height, diameter at breast height, the number of coppice stumps and biomass yield (Table 1).

Mimosine Toxicity – A Problem For Leucaena leucocephala used as Food

Despite the nutritional attributes shown, Leucaena is considered an invasive weed and its benefits are limited by the presence of a toxic non-protein free amino acid called mimosine; beta-(N-(3-hydroxy-4-oxypyridyl))(alpha)-amino propionic acid. The content of mimosine is relatively high in leaves compared to seeds. Mimosine is also known to be allelopathic, inhibiting the germination and growth of other horticultural and forestry species. The presence of mimosine varies among Leucaena species, growth rates, seasons and parts of the plant. There is about 2.03–4.89% mimosine in the dry matter of leaves, 0.68% in bark, 0.11% in xylem, 6-12% in the growing tips, 3-5% in young pods, 3.9-5% in seeds and 2% in green stems [13-17]. The content of mimosine in leaves decreases as the tree matures. Adeneye [18] reported a noticeable absence of mimosine in green and brown seed coats as well as empty brown pods, thus suggesting that diet supplemented using empty green and brown pods without any further treatment are safe for ruminant consumption.

In ruminants, mimosine is degraded to its immediate secondary metabolite; 3-hydroxy-4-1(H)-pyridone (3,4-DHP). There is also a certain endogenous plant enzyme present in leaves and seeds that is capable of catalyzing this conversion. The toxicity of Leucaena is believed to be from mimosine and 3,4-DHP, which will be further degraded into its isomer 2,3-dihydroxypyridine (2,3-DHP) in the rumen. Yet, conversion to these intermediates does not detoxify the toxicity. Generally, ruminants (cattle, sheep, and goats) are better at tolerating Leucaena than non-ruminants (horses, pigs and poultry) due to the presence of micro flora in the rumen. Structurally, mimosine is known to be a tyrosine analog that suppress tyrosinase and tyrosine decarboxylase. It is also recognized to be anti-peroxidase, inhibiting peroxidase and lactoperoxidase reactions that is, by interfering with the iodination of tyrosine, thus affecting the synthesis of T1, T2, T3 and T4 [19]. Findings showed that circulating DHP in blood inhibits metal-chelating enzymes, such that it forms complexes with Zn and Cu, or Fe leading to excretion and depletion of these metals (Figure 1). Typical symptoms associated with mimosine and 3,4-DHP toxicity include alopecia, loss of appetite, growth retardation, excessive salivation in cattle and buffalo, reduced fertility, goitre and death. However, it was observed that only the actively growing hair (proliferative phase) is affected rather than resting hair (keratinised phase) [20]. The prevalence of toxicity was believed to take effect with respect to the amount of Leucaena and the duration of consumption, where extended consumption causes a decrease in live weight instead of increasing it. In contrast, there is no reduction in milk and meat yield when consuming Leucaena leaf meal, reflecting no mimosine and DHP toxicity possibly due to the barrier between the blood and udder, thus making it safe for human consumption [21] (Table 2).

Table 2: Symptoms of Leucaena toxicity in animals.

Symptoms	Animals	References
Alopecia, loss of appetite, excessive salivation, poor breeding performance, thyroid hypertrophy, loss of body weight	Cattle, Buffalo, Sheep, Pig	[22-25]
Depression of T3 and T4 level	Cattle, Buffalo	[26, 27]
Ulceration	Cattle, Sheep	[27, 28]
Alopecia, infant mortality	Lemur	[29]
Reversible paralysis	Rat	[30]
Alopecia, rapid weight reduction, serious liver and kidney degenerative	Rabbit	[31, 32]
Reduced weight gains, egg mass and egg production	Chicken	[33]

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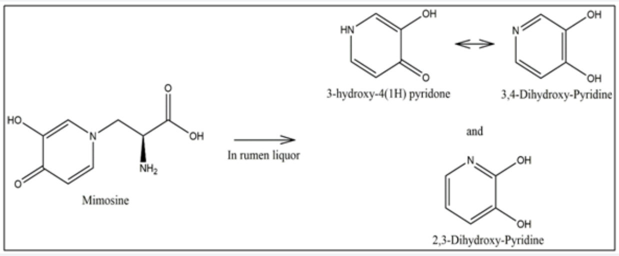

Figure 1: Degradation of Mimosine into its metabolites by ruminal micro organisms.

Mimosine Removal Techniques to Be Used on Food

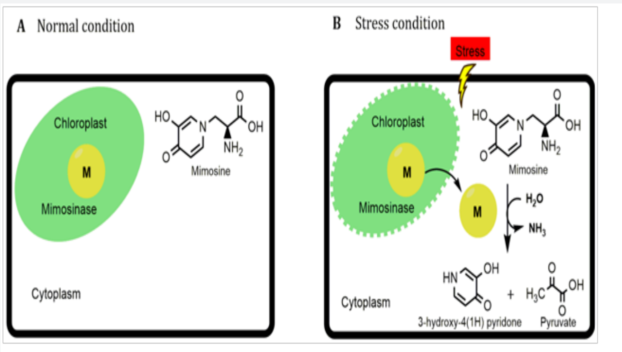

Figure 2: Illustration of Mimosine in cytoplasm and Mimosinase in chlorophyll of Leucaena plant. A. Normal condition. B. Stress condition.

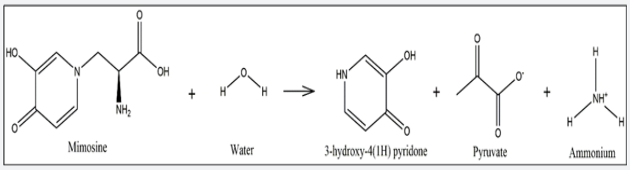

Figure 3: Illustration of balanced reaction of mimosine degradation catalyzed by the C-N lyase enzyme.

There are considerable methods that have been proposed to reduce the toxicity of Leucaena for ruminal feedstock. Factors such as leaf condition (fresh leaf and dry leaf) also play a role in determining the effectiveness of mimosine degradation. A study by [22] revealed that different removal methods significantly affect the mimosine content and its conversion. They found that the most effective approaches employed for mimosine degradation were by soaking fresh leaves with water at room temperature for 36 hours, soaking leaves at 60^{0}C in hot water for 24 hours and fermentation for 5 days for 40% Leucaema inclusion in the rabbit diet. The observed effect was pronounced as the temperature rises together with prolonged soaking. However, the macerated leaves gave only a slight increase in mimosine degradation. Other researchers, including [23] has affirmed that about 94% reduction of mimosine content and virtually all tannins in the leaf meal could be obtained when employing the drying–soaking-drying technique. It is speculated that instead of over expression of mimosinase at high temperature, the study found that the release of mimosinase and other enzymes to catalyse mimosine was due to the breakdown of chlorophyll as the temperature rises in the intact leaves, though enzyme efficiency decreases slightly as a consequence of denaturation. In nature, the utilization of mimosine was probably in the case of stress, for an energy source, when the nitrogen and carbon become scarce [24] (Figures 2 & 3).

Inactivation of mimosine toxicity also could be seen through the adaptation of animals, which is considered geographical. Studies found that goats in Hawaii experience no adverse effect of mimosine toxicity, but not goats in Australia. Owing to this, attempts have been made to detect the microbes responsible for the mimosine-degrading characteristic, which is known to be Synergistes jonesii; S. jonesii. They found a high level of 2,3 - DHP content in the urine and feces after inoculating S. jonesii in the ruminant stomach and this microbial population tended to persist several months once the Leucaena diet stopped. It is suggested a modification in diet, where rumen degradation of mimosine in sheep fed on a lucerne-oat diet is more rapid than that of sheep fed on lucerne hay only and the degradation process was achieved by the bacterial fraction rather than the protozoic fraction [25]. In term of mechanism, it was postulated that the mechanism was due to the naturally occurring enzymatic reaction from the plant, a mimosinase that degrades mimosine into 3-hydroxy-4-pyridone (3H4P), whereas others identified the mimosine-degrading enzyme in seedling extracts as a carbon-nitrogen (C-N) lyase that converted mimosine into 3,4-dihydroxypyridine (3,4DHP) and its by-products pyruvic acid, and ammonia.

Physico-chemical mimosine inactivation approaches such as inclusion of ferric chloride in rabbit rations exhibit no mimosine excreted in feces, while increased 3,4-DHP excretion in treated Leucaena leaf meal indicates that mimosine forms a chelate with Fe^{+++} ions, hence preventing the mimosine from being absorbed in the intestine; leading to substantially reduced toxicity symptoms. The result from treating Leucaena leaf with ferrous sulphate also shows a comparable result in growing pig rations, with the treated leaves being 20% of the whole diet [26]. Similarly, chelation between mimosine and copper was observed when given at 10mg kg^{-1} and higher, together with iron at 8g kg^{-1} in calves' diet [27]. Others found incorporating iodine in the goat diet increases the level of T4 (thyroxine) significantly, thus poses a possibility to alleviate the toxicity on the thyroid gland, although the result might be inconclusive due to the short period of time [28].

Researchers are now focusing on developing transgenic Leucaena that has a low mimosine content. A Leucaena clone was introduced by soaking the seedlings in ethyl methanesulphonate (EMS) at different concentrations prior to planting. The result showed 0.6% of EMS produced the lowest mimosine containing Leucaena (87.5% reduction). However, it appears that there is slight decrease in nutritive values of cloned Leucaena (18.69%) though the value still exceeds that alfalfa (14.83%) in crude

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protein [29,30] reported TAL1145, a Leucaena nodulating *Rhizobium* sp. strain, could degrade mimosine (Mid+) into 3-hydroxy-4-pyridone readily. The corresponding enzyme with a polypeptide of 45kDa; an amino transferase encoded by mid D gene provides the TAL1145 strain a competitive advantage over other *Rhizobium*, *Sinorhizobium* and *Bradyrhizobium* spp. Another study by [31] identified the first bacterial strain found in soil independently from the Leucaena tree that was capable of degrading mimosine (Pseudomonas species; P. putida STM 905) into carbon and nitrogen as an energy source. The gene responsible for the degradation activity possesses a molecular mass of 70 kDa and is believed to be more efficient than that of the *Rhizobium* sp. *Rhizosphere* strain that is capable of degrading mimosine [32-42].

Conclusion and Recommendation

L. leucocephala poses valuable benefits in various aspects from animal feedstock to biomass resources. However, the presence of mimosine and its intermediate; 3,4-DHP and 2,3-DHP limits the utilization of Leucaena. Abundant approaches have been made in order to overcome this limitation. However, thorough work is needed if possible to fully eliminate the toxicity in a time-effective manner, for *Leucaena* utilization to produce both bioproducts and bioenergy.

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