

Brief Review: The Negative Impact Of Mimosin in *L. leucocephala* in Ruminant Animals and Processing Methods to Reduce Poisoning Effects on Ruminant Livestock

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Abstract

Leucaena leucocephala, a high-quality ruminant feed, is essential for livestock production in the tropics, despite the presence of mimosine in the leaves. Mimosine, in high concentrations, can severely affect animal health and performance. Mimosine and its metabolites, 3-hydroxy-4-(1H)-pyridon (DHP), are toxic to ruminants and caused hair loss, slow growth, and oral ulceration, whereas DHP is goitrogenic because it is analogous to tyrosine so resulting in goiter. Mimosine and its metabolites are the main hindrance blocks for the utilization of *L.leucocephala* as animal feed. Characteristic signs of *L.leucocephala* toxicity are alopecia, anorexia, reduced weight gain, and weight loss, excessive salivation, esophageal lesions, enlarged thyroid and low circulating concentrations of thyroid hormones. Therefore, the research workers had tried to develop different methods to eliminate the toxicological effects of mimosine and its metabolites. This paper aims to briefly review the negative effects of mimosine from *L. leucocephala* and its processing to reduce the toxic effects on ruminants.

Keywords: *Leucaena leucocephala*, mimosine, toxicity, metabolites

Introduction

Forage plants such as grasses and legumes are basic food ingredients that provide nutritional and energy needs for ruminants. One of the legumes which are widely used as ruminant animal feed ingredients is Chinese Petai or Lamtoro (*Leucaena leucocephala*). *L. leucocephala* plants are drought tolerant and are widely distributed in subtropical and tropical zones in Southeast Asia and Africa, and are mostly planted to prevent and inhibit soil erosion and soil nutrient improvement (Brewbaker and Sorensson 1990; Hong et al., 2003). *Leucaena leucocephala* is a legume that has high palatability and nutritional value as a source of protein in animal feed (Blom, 1981; Andrew et al., 2000). However, its use as a food source has limitations because of the antinutrient

factors (ANF) contained in it. One of the antinutrients contained in forage is mimosine (Brahmachari, 2012). *L. leucocephala* has a high enough mimosine content that will be toxic if it is excessively consumed as ruminant feed (Ross and Springhall 1963; Shelton and Brewbaker 1994; Ramli et al., 2017).

Mimosine (*leucaenine* or *leucenol*) is an amino acid that is toxic and is mostly contained in leaves, pods, flowers and legume seeds from tropical to subtropical regions such as *Mimosa pudica* and *Leucaena leucocephala* (Jones et al., 1976; Hulman et al., 1978; Shelton and Dalzell, 2007; Pund et al., 2017). Mimosine and its metabolites, 3-hydroxy-4-(1H)-pyridone (DHP), are toxic to ruminants and can cause an acute poisoning (Hegarty et al., 1964a; D'Mello 2003; Chanchay and Poosaran, 2009; Dalzell et al., 2012). The

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toxic effects of mimosine include hair loss, slow growth, and oral ulceration, while DHP is goitrogenic because it is analogous to tyrosine so that it can cause goiters or goiter (Jones and Hegarty, 1984; Hammond, 1995; Reis et al., 1999). Clinical symptoms observed in mimosine poisoning are tongue ulceration, buccal cavity congestion, thinness, ear, eye lesions and thyroid hypertrophy in ruminants (Pachauri and Pathak, 1989; Ram et al., 1994; Vijayakumar and Srinivasan, 2018). Various methods have been used to reduce the risk of poisoning in ruminants, such as withering, drying, immersion in hot water, extraction, and fermentation (Ghatnekar et al., 1983; Vogt et al., 1986; Nursiwi et al., 2018). This paper aims to briefly review the negative effects of mimosine in *Leucaena leucocephala* and its processing to reduce the toxic effects on ruminants.

Nutrients in *L. leucocephala*

Leucaena consists of 53 species, classified into 10 species that have been known to date, but only *Leucaena leucocephala* has been widely used as animal feed, especially in the tropics (NAS, 1984; Brewbaker et al., 1985; Schultze-Kraft et al., 2018). *Leucaena leucocephala* is a type of shrub sized and a fast-growing legume, originating from Central America and has been widely used as animal feed in almost all tropical and subtropical countries (Nuttaporn and Naiyatat, 2009; Pandey and Kumar, 2013). *Leucaena leucocephala* has several common names such as lamtoro, White Lead tree, White Popinac, Jumbay,

Wild Tamarind, ipil ipil, subabul, and grasshoppers depending on the country they are growing in (Holm et al., 1979; Devi et al., 2013; Mandey et al., 2015; Mohamed et al., 2014).

All parts of *L. leucocephala* such as leaves, young stems, flowers, pods, including grain, can be consumed by livestock (Mendoza, 1975; Barros-Rodriguez et al., 2013). *Leucaena leucocephala* is an important source of animal feed because it is rich in protein, essential amino acids, minerals, carotenoids, and vitamins (Chen and Wang, 2010; Ayssiwede, et al., 2010; Zayed and Benedict, 2016). D'Mello and Thomas (1978) added that *Leucaena leucocephala* leaves and stems are rich in nutrients and fiber, making them a near-perfect ruminant feed ingredient, compared to alfalfa. The amino acid content of *Leucaena leucocephala* is comparable to soybean meal and fish meal (Ter Meulen et al., 1979). Siahaan (1982) in his research, stated that *L. leucocephala* leaves had the following composition: 34.5% dry weight, 21.5% crude protein, 49.5% extract material without nitrogen (BETN), 14.3% crude fiber, 6.5% fat, 6.28% ash, 2.7% calcium, and 0.17% phosphorus. According to D'Mello (1992), *L. leucocephala* contains: 24.2% crude protein, 7.5% ash, 2450 kcal / kg metabolic energy, 21.5% crude fiber, 1.68% calcium, and 0.21% phosphorus. While the form of *L. leucocephala* leaf flour contains: 88.2% dry matter, 21.8% crude protein, 15.1% crude fiber, 3.1% ash, 8.6% ether

extract and 50.7% BETN (Eniolorunda, 2011). Research by Yessirita (2012) showed that *L. leucocephala* leaf flour contains: 22.69% crude protein, 1.55% fat, 16.77% crude fiber, 11.25% ash, 1.92% Ca, 0.25% and P and 331.07 ppm β -carotene. The mineral content is strongly influenced by mineral elements in the soil so *L. leucocephala* can be a major source of calcium, phosphorus, and other minerals, although it may have sodium deficiency (D'Mello and Fraser, 1981; Akbar and Gupta, 1985). The nutrient content of *L. leucocephala* varies, influenced by location, variety, plant age, soil type, and season (Ghosh and Bandyopadhyay, 2007). The variation of the content causes the amount or volume of *L. leucocephala* leaves have to be adjusted, according to the conditions of each region, the age of the plant and the harvest season to minimize the negative impact of mimosine.

The results showed that *L. leucocephala* can substitute protein supplement products in dairy cows because it can increase milk production (Flores-Ramos, 1977; Flores et al., 1979). Rivera et al. (2018) stated that feeding *L. leucocephala* in dairy cows produced higher fat milk compared to basal grass-feeding added with concentrate. Research by Hulman et al. (1978) showed an increase in body weight of 0.85 kg/day in beef cattle fed *L. leucocephala* with the addition of urea molasses. Feeding *L. leucocephala* leaves in fresh or dried form in sheep increased body weight from 22.7 to 68.1 g/day

(Quintyne, 1987). Jones and Bunch (1995) in their research report, showed that feeding with *Brachiaria decumbens* grass and *L. leucocephala* leaves were able to increase milk fat and protein content in dairy cows compared to the provision of a single *Brachiaria decumbens*. Despite having a high nutritional value, *L. leucocephala* is not recommended for use as a single feed for ruminants due to the presence of mimosine and the high content of crude fiber (Halliday et al., 2013). Although it can be toxic to livestock, mimosine also has various anti-cancer benefits (Chang et al., 1999), antimicrobial (Mohammed et al., 2015), herbicide (Xuan et al., 2006), anthelmintic (von Son-de Fernex et al., 2015), insecticides (Nguyen et al., 2015), anti-inflammatory (Zayed and Benedict, 2016), and antioxidants (Chowtivanakul and Talubmook, 2012).

Mimosin Toxicity in Livestock

Mimosine [β -[N-(3-hydroxy-4-oxypyridyl)]- α -amino propionic acid] is a non-protein amino acid compound, widely contained in all parts of the *Mimosaceae* plant, including *L. leucocephala* (Brewbaker and Hylin, 1965; Tacon, 1979; Vogt et al., 1986; Wee and Wang, 1987). The main metabolite of mimosine is 3-hydroxy-4(1H)-pyridine or 3-4 DHP (D'Mello, 1992). Apart from being an excellent source of legume feed, *L. leucocephala* leaves and seeds contain several toxic substances such as mimosine and tannin which can limit their use in livestock. According to Hashiguchi and Takahashi (1977) and Dai et al. (1994),

mimosine has a role as a pyridoxal inhibitor, containing transaminases, tyrosine decarboxylase, several enzymes containing metals, cystathionine synthetase, and cystathionase. Puchala et al. (1996) stated that mimosine has the same structure as tyrosine and has been known to replace the amino acid. Replacement of these amino acids can cause the loss of enzymes and functional activity of proteins. Vickery and Vickery (1981) added that the toxicity of mimosine comes from the presence of -OH and -O in the pyridine ring that is known to be able to suppress enzymes that contain iron and can compete with tyrosine. The toxin power of mimosine may also be caused by acute antimitotic properties, inhibiting DNA synthesis, especially in cells that undergo rapid division, that can cause damage to organs in the body (Tsai and Ling 1971; Prasad and Paliwal 1989; Perry et al., 2005; Xuan et al., 2016).

In addition to being toxic to ruminants, mimosine is also toxic to non-

ruminant animals such as pigs (Echeverria et al., 2003), rabbits (Adejumo, 2006), fish (Hossain and Shikha 1997), poultry (D'Mello, 1992), and experimental animals such as mice (Wiratmini et al., 2014). *L. leucocephala* is not recommended for use as the main feed for non-ruminant animals, and its use is limited to 5% to 10% of the dry weight (Rushkin, 1984). The toxic substances for ruminant animals are mimosine and DHP as metabolites (Ram et al., 1994). *L. leucocephala* leaves contain mimosine content that varies between 2.3 to 12%, depending on the variety (Gampawar et al., 1988; Kumar, 2003). Variation in the content of mimosine in *L. leucocephala* makes us more careful in applying it as a forage source of protein for ruminants to avoid the negative effects of mimosine. Table 1 shows the various content of *L. leucocephala* mimosine from various sources.

Table 1. Various content of *L. leucocephala* mimosine from various sources.

Sources	Mimosine contents (%) of <i>L. Leucocephala</i>	References
Young leaf	9	Hegarty <i>et al.</i> , 1964b
Young leaf	10	Brewbaker and Hylin, 1965
Seed	3,61	Jones, 1979
Leaf	5.75	Chou and Kuo, 1986
Seed	5.04	Gampawar <i>et al.</i> , 1988
Leaf	2,14	Widiyastuti, 2001
Leaf flour	5.56	Kumar, 2003
Seed	10,00	Meena-Devi <i>et al.</i> , 2013
Seed	2,10	Benjakul <i>et al.</i> , 2014
Leaf	1,60	Zayed <i>et al.</i> , 2014
Leaf	8,47	Ilham <i>et al.</i> , 2015
Leaf bud	14,7	Honda and Borthakur, 2019

The high content of mimosine causes poisoning if ruminants are fed with *L. leucocephala* containing more than 30% dry matter (Jones and Hegarty, 1984). However, research in Mexico showed a contrary result because ruminants could tolerate intake of *L. leucocephala* in the proportion of 50-60% without showing symptoms of poisoning (Ruiz-González et al., 2011; Contreras-Hernández et al., 2013). According to Hegarty et al. (1964a), rumen microflora may play a role in the degradation of mimosine to DHP. This statement is supported by the results of in vitro research by Aung et al. (2011), which stated that the bacteria *Streptococcus bovis* and *Klebsiella spp* could degrade mimosine to DHP, although in vivo has not been proven yet. Lowry et al. (1983) state that endogenous enzymes in *L. leucocephala* seeds are also able to degrade mimosine to DHP, pyruvate, and NH₃.

Jones's research report (1981) showed interesting results because mimosine poisoning did not occur when *L. leucocephala* was given as a goat or cow feed in Hawaii and Indonesia, proved by the low levels of DHP in these animals. Initially, Hegarty et al. (1964) and D'Mello (1992) indicated that mimosine metabolites 3,4-dihydroxypyridine (3,4 DHP) could be degraded by rumen microflora to 2,3-dihydroxypyridine (2,3 DHP). The final result of the 2,3-dihydroxypyridine (2, 3 DHP) degradation in the rumen is propionic acid (Rincón et al., 2003). This was confirmed by research by Jones and

Megarritty (1983) who discovered the existence of gram-negative bacteria of goats from Hawaii, which was able to degrade mimosine and DHP in the rumen. Subsequently, research carried out in the 1980s succeeded in isolating and identifying gram-negative bacterial species from the rumen of the Goat from Hawaii, later called *Synergistes jonesii*. Inoculums of these species were put in Australian cattle rumen in 1983, proven that it could reduce the toxic effects of mimosine by degrading into non-toxic metabolites (Jones and Megarritty 1986; Allison et al., 1992; Klieve et al., 2002). The results of Hammond's (1995) study also showed that susceptible animals could be given the ability to decrease 3,4 DHP and 2,3 DHP in a week by giving *Synergistes jonesii*. Quirk et al. (1988) strengthen the results of the above study, when *S. jonesii* was not present in the rumen, DHP as a mimosine derivative was not degraded and caused adverse toxic effects.

Clinical Symptoms of Mimosin Poisoning in Ruminants

Mimosine poisoning can be subclinical or clinical. Symptoms often appear after 2-4 months of *L. leucocephala* consumption (Quirk et al., 1988; Dalzell et al., 2012). Clinical symptoms of mimosine poisoning in cattle are characterized by alopecia, anorexia, weight loss, hypersalivation, lesions in the esophagus, abortion, low fertility, neonatal death, enlarged thyroid enlargement, and low thyroid hormone concentration (Jones,

1979; Holmes et al., 1981; Jones and Hegarty 1984; Vijayakumar and Srinivasan, 2018). Symptoms of poisoning in sheep can be in the form of non-optimal hair growth, hemorrhagic cystitis. Daily intake of mimosine 0.2-0.3 g/BW/day causes loss of the fleece, weight loss, mumps, neonatal death, and esophageal ulcers (Prasad and Paliwal, 1989; Prasad, 1988). Alopecia, anorexia, and weight loss are indicated by cattle that consume *L. leucocephala* for 1-10 months (Jones et al., 1978). Mimosine poisoning symptoms are various, despite many similarities, depending on the type of ruminant animal that consumes them. Clinical symptoms in nursing cows indicate salivation, thyroid enlargement, and weight loss (Hamilton et al., 1968). Research by Peixoto et al. (2008) showed the presence of diffuse alopecia in goats consuming *L. leucocephala* as basal feed for 4 months.

According to Lowry et al. (1983), mimosine poisoning can also appear and last for a short time. This can occur when ruminants consume it for the first time or after long periods of not consuming but then given in feed. This is due to the degradation of mimosine to DHP which occurs through the post-consumption hydrolase activity of plants and many endogenous rumen

microbes that can quickly and easily reduce the majority of mimosine consumed to become DHP within 2 weeks from the first giving (O'Reagain et al., 2014). Dominguez-Bello and Stewart (1991) found that gram-positive bacteria that form *Clostridium* strain 162 spores in the rumen were able to degrade 3, 4 DHP and 2, 3 DHP into normal metabolites. Seeing the conditions above, it can be concluded that with the right treatment strategy, mimosine can be reduced or even eliminated, so that it does not endanger the animals that consume it.

Mimosine Content Reduction Methods

Mimosine and DHP as its metabolites contained in *L. leucocephala* are toxic substances, so many research studies are carried out to reduce their content through biological and physicochemical techniques to increase the nutritional value of these plants. Currently, there are also many methods of reducing the content of mimosine in *L. leucocephala* by using chemicals such as FeCl₃ and HCl or enzymes derived from ruminant microorganisms that can degrade mimosine (Tawata, 1990; Soedarjo and Borthakur, 1996). Table 2 shows various methods of reducing the mimosine content of *L. leucocephala*.

Table 2. Methods for reducing mimosine content in *L. leucocephala*

Materials	Methods	References
Leaf	Extraction with 0.05 N CH ₃ COONa	Tacon, 1979
Leaf	Soaking	Ter Meulen <i>et al.</i> , 1979
Leaf	Cooking	Benge and Curran, 1981
Seed	Washing, soaking, boiling	Whiting, 1982
Leaf	Washing with <i>Sodium acetate</i> 0.05N	Tawata <i>et al.</i> , 1986
Flour	Soaking for 24 hours	
Leaf	Combination between washing, soaking, and cooking	Padmavathy and Shodha, 1987
Leaf	Soaking with hot water	Wee and Wang, 1987
Leaf	Heating using autoclave	Mali <i>et al.</i> , 1990
Leaf, seed, and stem	Soaking and boiling	Soedarjo and Borthakur, 1996
Leaf	60°C heating for 24 hours, then soaked in the hot water for 72 hours in room temperature, then dried at 60° C for 48 hours	Chanchay and Poosaran, 2009
Leaf	Heating 70°C for 15 minutes	Laconi and Widiyastuti, 2010
Seed	Soaking using <i>ethyl methanesulphonate</i> (EMS)	Mohamed <i>et al.</i> , 2014
Leaf	Extraction using ethyl acetate for 8 hours	Ilham <i>et al.</i> , 2015
Seed	Soaking, Boiling, and fermentation	Nursiwi <i>et al.</i> , 2018

Table 2 shows that the process of reducing mimosine content in leaves, stems, and seeds can be done easily and does not require high costs. According to Soedarjo and Borthakur (1996), soaking and boiling is the simplest method but can significantly reduce the content of mimosine. Some methods that are likely to require higher costs and more complicated workings are soaking with ethyl acetate, ethyl methane sulphonate, washing, and extraction with Sodium acetate 0.05N (Benjakul *et al.*, 2014).

Some methods carried out by researchers are described in the paper below. Tacon (1979) stated, the leaching of *L. leucocephala* leaves using saltwater, various acidic, and basic solutions for 24 hours at 25°C can significantly bring down

the concentration of mimosine. Further, the most effective extraction was by CH₃COONa 0.05 N, because it did not decrease much of the important nutrients. Although it does not significantly reduce the important nutrient content, the use of CH₃COONa 0.05 N is still difficult to apply in small scale livestock areas, which are mostly located in rural areas. The low costs method that easily applied is soaking and boiling. Extraction by soaking leaves in fresh water for 36 hours can reduce mimosine levels, up to 90%, increasing the nutritional value of *L. leucocephala* leaf flour (Wee and Wang, 1987). Boiling until boiling removes mimosine in *L. leucocephala* leaves and seeds, but can significantly reduce the dissolved protein content

(Nuttaporn and Naiyatat, 2009; Agbo et al., 2017).

Drying *L. leucocephala* leaves at 60°C for 24 hours, followed by immersion in water for 72 hours at room temperature, and drying again at 60°C for 48 hours can reduce the levels of mimosine from 4.35% to 0.22% (Chanchay and Poosaran 2009). Soaking *L. leucocephala* seeds in 80°C water for 3-4 minutes, followed by soaking in water at room temperature for 12 hours or soaking in concentrated sulfuric acid for 15-30 minutes, is a very useful initial processing method before the germination process (Padma et al., 1994). Dry heating of 1000°C can reduce the content of mimosine 9.45-14.52%. Heating 1000°C with 70% humidity can effectively decrease the content of mimosine to 50% (Akbar and Gupta, 1985). Different research results obtained by Atreja et al. (1990) stated dry heating does not influence the quality of *L. leucocephala*. These results are likely due to the research carried out, concerning that not only of the ANF aspect but also including the quality of the overall nutritional value after heat processing.

Other methods to reduce mimosine in *L. leucocephala* are physicochemical and biological. Treatment with supplementation of several types of mineral salts, such as iron, aluminum, copper, and calcium in *L. leucocephala* leaf flour has been reported to detract the toxic effects of mimosine due to the chelating effect, so that excretion occurs through feces (Ram et al., 1994; Gupta and Atreja, 1997). The above methods are

efforts to bring down the negative impact of mimosine on ruminants. Various methods give us many choices that we can adjust with the conditions of each farm area in utilizing *L. leucocephala* as a safe feed ingredient for ruminants.

Conclusion

L. leucocephala is a high nutritional value legume if it is given as a ruminant animal feed. However, *L. leucocephala* utilization as a ruminant animal feed needs to be limited, because the presence of ANF mimosine can cause negative effects if it is given in excessive amounts for a long time. The most common negative effects are alopecia, anorexia, weight loss, hypersalivation, and thyroid enlargement. Many methods to decrease the content of mimosine in *L. leucocephala* can be done and are expected to cut down the risk of poisoning in livestock. In the end, the method of reducing the content of mimosine depends on the ability of the farmer's economy to process it. The simplest and cheapest method is drying, washing, and soaking in freshwater.

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