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

Yanuartono*) , Soedarmanto Indarjulianto, Alfarisa Nururrozi, Slamet Raharjo, Hary Purnamaningsih
Department of Internal Medicine, Faculty of Veterinary Universitas Gadjah Mada

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)-piridon (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

*) Corresponding author:
E-mail:yanuartono20@yahoo.com

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 (Chowtivannakul 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 *L. Leucocephala***	**References**
Young leaf	9	Hegarty *et al.,* 1964b
Young leaf	10	Brewbaker and Hylin, 1965
Seed	3,61	Jones, 1979
Leaf	5.75	Chou and Kuo, 1986
Seed	5.04	Gampawar *et al.,* 1988
Leaf	2,14	Widiyastuti, 2001
Leaf flour	5.56	Kumar, 2003
Seed	10,00	Meena-Devi *et al.,* 2013
Seed	2,10	Benjakul *et al.,* 2014
Leaf	1,60	Zayed *et al.,* 2014
Leaf	8,47	Ilham *et al.,* 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 NH_3.

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 Megarrity (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 Megarrity 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 $FeCl_3$ 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 CH3COONa	Tacon, 1979
Leaf	Soaking	Ter Meulen *et al.,* 1979
Leaf	Cooking	Benge and Curran, 1981
Seed	Washing, soaking, boiling	Whiting, 1982
Leaf	Washing with *Sodium acetate* 0.05N	Tawata *et al.,* 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 *et al.,* 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^{0}C for 15 minutes	Laconi and Widiyastuti, 2010
Seed	Soaking using *ethyl methanesulphonate* (EMS)	Mohamed *et al.,* 2014
Leaf	Extraction using ethyll acetate for 8 hours	Ilham *et al.,* 2015
Seed	Soaking, Boiling, and fermentation	Nursiwi *et al.,* 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 CH_3COONa 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 CH_3COONa 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.

References

Adejumo, D. 2006. Performance and serum chemistry of rabbits fed graded levels of cassava peels, Leucaena leucocephala and Gliricidia sepium leaves based diets. *Global J Pure Appl Sci.*12:171-175

Agbo, A.N., J.K. Balogun, S.J. Oniye, and J. Auta. 2017. Effect of different processing methods on nutritional composition of Leucaena leucocephala (Lam De Wit) leaves as inclusion in Fish Feed. *J. Appl. Sci. Environ. Manage*. 21 (4): 719-725

Akbar, M.A., and P.C. Gupta. 1985. Proximate composition and tannin and mineral contents of various plant parts of subabul (Leucaena leucocephala). *Indian J Anim Sci*.55:808-812.

Allison, M., W. Mayberry, C. McSweeney, and D. Stahl. 1992. Synergistes jonesii, gen. nov., sp. nov.: a rumen bacterium that degrades toxic pyridinediols. *Systematic and Applied Microbiology*. 15: 522-529.

Andrew, W.M., M.J. Williams, M.J. Allison, C.C. Chase, C.G. Chambliss, R.S. Kalmbacher, and W.E. Kunkle. 2000. Detection of Synergistes jonesii in cattle and sheep feces. *Proceedings of the 37th North American Alfalfa Improvement Conference*. (Ed. M Phillips) American Forage & Grassland Council: Madison, WI. 165–168.

Atreja, P.P., R.C. Chopra, and A. Chhabra. 1990. *Report on Project Entitled to Test the Effect of Anti-Metabolites*. NDRI, Karnal.

Aung, A., U. Ter Meulen, F. Gessler, and H. Böhnel, 2011. Isolation of Mimosine Degrading Bacteria from Rumen Juice and Mass Production by Göttingen Bioreactor Technology. *Journal of Agricultural Science and Technology*. 1: 764-772

Ayssiwede, S.B., A. Dieng, C. Chrysostome, W. Ossebi, J.L. Hornick, and A. Missohou. 2010. Digestibility and Metabolic Utilization and Nutritional Value of Leucaena leucocephala (Lam.) Leaves Meal Incorporated in the Diets of Indigenous Senegal Chickens. *International Journal of Poultry Science*. 9 (8): 767-776

Barros-Rodríguez, M., J. Solorio-Sánchez, C. Sandoval-Castro, A.V. Klieve, E.B. Briceno-Poot, L. Ramirez-Aviles, and R. Rojas-Herrera. 2013. Effect of two intake levels of Leucaena leucocephala on rumen function sheep. *Trop Grasslands-Forrajes Tropicales*. 1:55–57.

Benge, M.D., and H. Curran. 1981. The use of Leucaena for soil erosion control and fertilization. In: Benge MD, ed. *Leucaena leucocephala: a tree that defies the woodcutter*. Washington, DC: Office of Agriculture, Development Support Bureau, Agency for International Development, 1981;Section 6:1-13.

Benjakul, S., P. Kittiphattanabawon, P. Sumpavapol, and S. Maqsood. 2014. Antioxidant activities of lead (Leucaena leucocephala) seed as affected by extraction solvent, prior dechlorophyllisation and drying methods. *J Food Sci Technol.* 51(11):3026–3037.

Blom, P.S. 1981. Leucaena, a promising versatile leguminous tree for the tropics. *International Trees and Crops Journal.* 1: 221-236.

Brahmachari, A.K. 2012. Evaluation of Mimosine Toxicity in Goat and Buffalo Erythrocyte System in vitro. *Explor. Anim. Med. Res*. 2(1): 66-69

Brewbaker, J.L., and J.W. Hylin. 1965. Variation in mimosine content among Leucaena species and related Mimosaceae. *Crop Science* 5(4): 348-349.

Brewbaker, J.L. and C.T. Sorensson. 1990. New tree crops from interspecific Leucaena hybrids. In: Janick, J. and Simon, J.E. (eds). *Advances in New Crops*. Timber Press, Portland, pp. 283-289.

Brewbaker, J.L., N. Hegde, E.M. Hutton, R.J. Jones, J.B. Lowry, F. Moog, and R. Van den Beldt. 1985. *Leucaena - Forage Production and Use*. Nitrogen Fixing Tree Association, Waimanalo, Hawaii.

Chanchay N., and N. Poosaran. 2009. The reduction of mimosine and tannin contents in leaves of Leucaena leucocephala. *Asian Journal of Food* *and Agro-Industry*. Special Issue : S137-S144.

Chang, H.C., T.H. Lee, L.Y. Chuang, M.H. Yen, and W.C. Hung. 1999. Inhibitory effect of mimosine on proliferation of human lung cancer cells is mediated by multiple mechanisms. *Cancer Lett* 145: 1–8.

Chen, C., and Y. Wang. 2010. Polyprenol from the whole plants of Leucaena leucocephala. *J Environ Prot.*1:70-72.

Chou, C.H., and Y.L. Kuo. 1986. Allelopathic research of subtropical vegetation in Taiwan : III. Allelopathic exclusion of understory byLeucaena leucocephala (Lam.) de Wit. *J Chem Ecol.* 12(6):1431-1448.

Chowtivannakul, S., and C. Talubmook. 2012. Antioxidant and antidiabetic activities of leaf and seed extracts from Leucaena leucocephala. (Lam.) de Wit. *Proceeding of NATPRO 4*. Chiang Mai, Thailand; 2012. 356-359.

Contreras-Hernández, M., N. Ruz-Ruiz, E. BriceñoPoot, L. Ramírez-Avilés, A. Ayala-Burgos, A. Pérez-Aguilar, J. Solorio-Sánchez, and J. Ku-Vera, 2013. Urinary excretion of mimosine metabolites by hair sheep fed foliage of Leucaena leucocephala. *Proceedings 22nd International Grassland Congress*. Australia.

D'Mello, J.P., and D. Thomas. 1978. The nutritive value of dried leucaena leaf-meal from Malawi: studies with young chicks. *Trop. Agric.* (Trinidad) 55:45

D'Mello, J.P.F., and K.W. Fraser. 1981. The composition of leaf meal from Leucaena leucocephala. *Trop Sci.* 23: 75 -78.

D'Mello, J.P.F. 2003. *Amino acids in animal nutrition* 2nd. Wallingford (UK) : CABI Publishing.

D'Mello, J.P.F. 1992. Chemical constraints to the use of tropical legumes in animal nutrition. *Anim. Feed Sci. Technol*. 38: 237-261.

Dai, Y., B. Gold, J.K. Vishwanatha, and, S.L. Rhode. 1994. Mimosine inhibits viral DNA synthesis through ribonucleotide reductase. *Virology*. 205: 210-216

Dalzell, S.A., D.J. Burnett, J.E. Dowsett, V.E. Forbes, and H.M. Shelton. 2012. Prevalence of mimosine and DHP toxicity in cattle grazing Leucaena leucocephala pastures in Queensland, Australia. *Animal Production Science*. 52: 365–372.

Devi, V.M., V.N. Ariharan, and P.N. Prasad. 2013. Nutritive value and potential uses of Leucaena Leucocephala as Biofuel–a mini review. *Res J Pharm Biol Chem Sci*.4:515-21.

Dominguez-Bello, M.G., and C.S. Stewart. 1991. Characteristics of a rumen Clostridium capable of degrading mimosine, 3(OH)-4-(1H)-pyridone and 2,3 dihydroxypyridine. *Syst. Applied Microbiol*. 14: 67-71

Echeverria, V., R. Belmar, J. Ly, and R.H. Santos-Ricalde. 2003. Effect of Leucaena leucocephala leaf meal treated with acetic acid or sodium hydroxide on apparent digestibility and nitrogen retention in pig diets. *Anim Feed Sci Technol*.101:151-159.

Eniolorunda, O.O. 2011. Evaluation of biscuit waste meal and Leucaena leucocephala leaf hay as sources of protein and energy for fattening "yankassa" rams. *African J. of Food Sci*. 5 (2):57-62.

Flores, J., T.H. Stobbs, and D.J. Minson. 1979. The influence of the legume Leucaenaleucocephala and formal-caseia on the production and composition of milk from grazing cows. *J Agric Sci*. 92:351-357.

Flores-Ramos. J.F. 1977. Leucaena leucocephala for milk production: effect of supplementation with Leucaena on cows grazing grass pastures. *Trop Anim Prod*. 4(1): 55-60

Gampawar, A.S., V.M. Garantiwar, and S.S. Bhaiswar. 1988. Effect of feeding subabul (Leucaena leucocephala seed as a part of protein supplementation on growth in Sahiwal X Jersey calves. *India J. Anim. Nutr*. 5: 240-243.

Ghatnekar, S.D., D.G. Auti, and V.S. Kamat. 1983. *Feeding Leucaena to Mozambique tilapia and Indian major carp*. In : IDRC 2nd Int. Workshop on Leucaena Research in the Asia Pacific Region, Singapore, IDRC 211, Unipub, New York. 61-63.

Ghosh, M.K. and S. Bandyopadhyay. 2007. Mimosine Toxicity-A Problem of Leucaena Feeding in Ruminants . *Asian Journal of Animal and Veterinary Advances*. 2: 63-73.

Gupta, H.K. and P.P. Atreja. 1997. Influence ferric chloride treated Leucaena leucocephala leaf meal on metabolism of mimosine and 3-hydroxy 4 (1H) pyridine in growing rabbits. *Proc. VIII Animal Nutrition Resource Workers Conference.* Chennai. 139.

Halliday, M.J., J. Padmanabha, C.S. McSweeney, G. Kerven, and H.M. Shelton. 2013. Leucaena toxicity: a new perspective on the most widely used forage tree legume. *Tropical Grasslands* 1: 1-11

Hamilton, R.I., L.H. Donaldson, and L.J. Lambourne. 1968. Enlarged thyroid gland in calves born to heifers fed on sole diet of Leucaena leucocephala. Aust. Vet. J. 44: 484-484.

Hammond, A.C. 1995. Leucaena toxicity and its control in ruminants. *J. Anim. Sci*. 73: 1478-1492.

Hashiguchi, H., and H. Takahashi. 1977. Inhibition of two copper-containing enzymes, tyrosinase and dopamine β-hydroxylase, by L-Mimosine. *Molecular Pharmacology*. 13: 362-367

Hegarty, M.P., R.D. Court, and P.M. Thorne. 1964b. The determination of mimosine and 3,4-dihydroxypyridine in biological material. *Aust. J. of Agric. Res.* 15:168−179

Hegarty, M.P., P.G. Schinckel, and R.D. Court. 1964a. Reaction of sheep to the consumption of Leucaena glauca Benth. and to its toxic principle mimosine. *Aust. J. Agric. Res.* 15: 153-167

Holm, L., J.V. Pacho, J.P. Herberger, and D.L. Plucknett. 1979. *A geographical atlas of world weeds*. Malabar, Florida: Krieger Publishing Company.

Holmes, J.H.G., J.D. Humphrey, E.A. Walton, and D.J. Oshea. 1981. Cataracts, goitre and infertility in cattle grazed on an exclusive diet of Leucaena leucocephala. *Australian Veterinary Journal.* 57: 257–261.

Honda, M.D.H., and D. Borthakur. 2019. Mimosine concentration in Leucaena leucocephala under various environmental conditions. *Tropical Grasslands-Forrajes Tropicales*. 7(2):164–172.

Hong, N.H., T.D. Xuan, E. Tsuzuki, H. Terao, M. Matsuo and T.D. Khanh. 2003. Screening for allelopathic potential of higher plants from Southeast Asia. *Crop Prot.* 22: 829–836.

Hossain, M., and F.H. Shikha. 1997. Apparent protein digestibility coefficients of some low protein ingredients for African catfish, Clarias gariepinus. *Bangladesh J Zool*. 25:77-82.

Hulman, B., E. Owen, and T.R. Preston. 1978. Comparison of Leucaena leucocephala and Groundnut Cake as Protein Sources for Beef Cattle Fed ad libitum Molasses/Urea in Mauritius. *Tropical Animal Production*. 3: 1-8.

Ilham, Z., H. Hamidon, N.A. Rosji, N. Ramli, and N. Osman. 2015. Extraction and Quantification of Toxic Compound Mimosine from Leucaena leucocephala Leaves. *Procedia Chemistry.* 16: 164-170.

Jones, R., and G.A. Bunch. 1995. Long-term records of legume persistence and animal production from pastures based on Safari Kenya clover and leucaena in subtropical coastal Queensland. *Tropical Grasslands*. 29:74–80.

Jones, R.J., and M.P. Hegarty. 1984. The effect of different proportions of Leucaena leucocephala in the diet of cattle on growth, feed-intake, thyroid-function and urinary-excretion of 3-hydroxy-4(1h)-pyridone. *Aust. J. Agric. Res*. 35: 317–325.

Jones, R.J. 1981. Does ruminal metabolism of mimosine explain the absence of leucaena toxicity in Hawaii ?. *Australian Veterinary Journal*. 57:55−56.

Jones, R.J. and R.G. Megarrity. 1983. Comparative toxicity responses of goats fed on Leucaena leucocephala in Australia and Hawaii. *Aust. J. Agric. Res*. 34:781−790.

Jones, R.J. and R.G. Megarrity. 1986. Successful transfer of DHPdegrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. *Australian Veterinary Journal.* 63:259−262.

Jones, R.J. 1979. The value of Leucaena leucocephala as a feed for ruminants in the tropics. *World Anim. Review*. 31(1):13-23.

Jones, R.J. C.G. Blunt, and B.I. Nurnberg. 1978. Toxicity of Leucaena leucocephala. The effect of iodine and mineral supplements on penned steers fed a sole diet of Leucaena. *Australian Veterinary Journal*.54: 387-392.

Jones, R.J., C.G. Blunt, and J.H.G Holmes. 1976. Enlarged thyroid glands in cattle grazing leucaena pastures. *Tropical Grasslands*. 10:113-116.

Klieve, A.V., D. Ouwerkerk, A. Turner, and R. Roberton. 2002. The production and storage of a fermentor-grown bacterial culture containing Synergistes jonesii, for protecting cattle against mimosine and 3-hydroxy-4(1H)-pyridone toxicity from feeding on Leucaena leucocephala. *Aust. J.Agric.Res.* 53:1−5.

Kumar, R. 2003. Anti-nutritive factors, the potential risks of toxicity and methods to alleviate them. http://www.fao.org/docrep/003/t0632e/T0632E10.htm. 4 August-2019

Laconi, E.B., and T. Widiyastuti. 2010. Kandungan Xanthofil Daun Lamtoro (Leucaena leucochepala) Hasil detoksifikasi Mimosin secara Fisik dan Kimia. *Jurnal Media Peternakan*. 33(1): 50-54.

Lowry, J.B., Maryanto and B. Tangendjaja, 1983. Autolysis of mimosine to 3-hydroxy-4-1(h)pyridone in green tissues of Leucaena leucocephala. *J. Sci. Food Agric*. 34: 529-533.

Mali, J.M., L.S. Kute, N.D. Jambhale, and S.S. Kadam. 1990. Effect of leaf processing on antinutrients in leucaena seeds. *Indian J. Anim. Sci.* 60: 385-388.

Mandey, J.S., N.D. Kumajas, J.R. Leke and M.N. Regar. 2015. Manfaat Daun Lamtoro (Leucaena leucocephala Dalam Pakan Ayam Pedaging diukur dari Penampilan Produksi. Jurnal Zootek ("Zootrek" Journal). 35 (1) : 72-77

Meena-Devi, V.N., VN. Ariharan, and N.P. Prasad. 2013. Nutritive Value and Potential Uses of Leucaena Leucocephala as Biofuel – A Mini Review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 4(1): 515-521

Mendoza, R.C. 1975. Herbage crude protein and digestible dry matter yield of Ipil-Ipil (Leucaena latisilique Cv. Peru). *Hedge Rows-Animal scientific Convention of the Philippines Society of Animal Science.*

Mohammed, R.S.,, S.S. El Souda, H.A.A. Taie, M.E. Moharam, and K.H. Shaker. 2015. Antioxidant, antimicrobial activities of flavonoids glycoside from Leucaena leucocephala leaves. *Journal of Applied Pharmaceutical Science* .5 (06): 138-147.

Mohamed, Z.Z., B.A. Fasihuddin, A.Z. Mohamed, H. Wei-Seng, and P. Shek-Ling. 2014. The reduction of mimosine content in Leucaena leucocephala (petai belalang) leaves using ethyl methanesulphonate (EMS). *Arch. Appl. Sci. Res.* 6 (4):124-128

NAS. 1984. Agroforestry in the West African Sahel. BOSTID, National Academy of Sciences: Washington, DC, USA.

Nguyen, B.C.Q., J. Chompoo, and S. Tawata. 2015. Insecticidal and nematicidal activities of novel mimosine derivatives. *Molecules* 20: 16741–16756.

Nursiwi, A., D. Ishartani, A.M. Sari and K. Nisyah. 2018. Study on Leucaena leocochepala seed during fermentation : sensory characteristic and changes on anti nutritional compounds and mimosine level *IOP Conf. Series: Earth and Environmental Science 102.*

Nuttaporn, F., and P. Naiyatat. 2009. The reduction of mimosine and tannin content in leaves of Leucaena leucocephala. *Asian Journal of Food and Agro-Industry*. Special issue. S137-S144.

O'Reagain, J.H., S.R. Graham, S.A. Dalzell, and H.M. Shelton. 2014. Rates of Urinary Toxin Excretion in Unprotected Steers Fed Leucaena leucocephala. *Tropical Grasslands – Forrajes Tropicales*. 2: 103–105.

Pachauri, V.C. and P.S. Pathak. 1989. Effect of feeding Leucaena leucocephala in combination with hybrid napier on growth and nutrient utilization in crossbred calves. *Indian J. Anim. Nutr.* 6: 158-161.

Padma, V., G. Satyanarayana, and B.M. Reddy. 1994. Effect of scarification treatments on the germination ofLeucaena leucocephala, Albizzia lebbeck and Samanea samon. *Seed Research*. 22:54-57.

Padmavathy, P. and S. Shobha. 1987. Effect of processing on protein quality and mimosine content of subabul (Leucaena leucocephala). *J. Food Sci. Technol.* 24: 180-182.

Pandey, V.C., and A. Kumar. 2013. Leucaena leucocephala: an underutilized plant for pulp and paper production. *Genet Resour Crop Evol.* 60:1165–1171

Peixoto, P.V., T.N. França, B.M. Cunha, D.V.A.M. Tavares, and M.F. Brito. 2008. Spontaneous poisoning by Leucaena leucocephala in a goat from Rio de Janeiro State, Brazil. *Ciência Rural.* 38 (2):551-555.

Perry, C., R. Sastry, I.M. Nasrallah, and P.J. Stover. 2005. Mimosine attenuates serine hydroxymethyltransferase transcription by chelating zinc − Implications for inhibition of DNA replication. *Journal of Biological Chemistry.* 280:396−400.

Prasad, J., and O.P. Paliwal. 1989. Pathological changes in experimentally induced Leucaena toxicity in lambs. *Indian Vet. J.* 66: 711-714.

Prasad, J. 1988. Clinicopathological aspects of experimental Leucaena toxicity in lambs. *Indian J. Anim. Sci*. 58: 1161-1166.

Puchala, R., J.J. Davis, and T. Sahlu. 1996. Determination of mimosine and 3,4 dihydroxypyridine in milk and plasma of goats. *J Chromatogr B Biomed Appl*. 685(2):375-378.

Pund, G., D.C. Kothari, and P.V. Thorat. 2017. Lab scale extraction of mimosine from leucaena leucocephala leaves. *International Research Journal of Engineering and Technology*. 4 (6):642-646

Quintyne, R.C. 1987. Utilization of Leucaena leucocephulu as a Livestock Feed in Barbados. I st Caribbean Leucaena Industries Symp., Kingston, Jamaica.

Quirk, M.F., J.J. Bushell, R.J. Jones, R.G. Megarrity, and K.L. Butler. 1988. Liveweight gains on leucaena and native grass pastures after dosing cattle with rumen bacteria capable of degrading DHP, a ruminal metabolite from leucaena. *Journal of Agricultural Science* 111: 165–170.

Ram, J.J., P.P. Atreja, A. Chhabra, and R.C. Chopra. 1994. Mimosine degradation in calves fed sole diet of Leucaena leucocephala in India. *Trop. Anim. Health Prod*. 26: 199-206.

Ramli, N., A. A. Jamaludin, and Z. Ilham. 2017. Mimosine Toxicity in Leucaena Biomass: A Hurdle Impeding Maximum use for Bioproducts and Bioenergy. Int J Environ Sci Nat Res 6(4):1-5

Reis, P.J., R. Puchala, T. Sahlu, and A.L. Goetsch. 1999. Effects of Mimosine and 2,3-Dihydroxypyridine on Fiber Shedding in Angora Goats. *J. Anim. Sci.* 77:1224–1229.

Rincón, M.T., M.G. Domínguez-Bello, M.J. Allison, R. Romero, Y. D'Sanctis, and F. Michelangeli. 2003. Biochemical aspects of mimosinederived toxic pyridinediols degradation by the rumen bacterium Synergistes jonesii. *Revista de la Facultad de Ciencias Veterinarias, FCV-LUZ.* 44: 17-35

Rivera, J.E., J. Chará, E. Murgueitio, J. J. Molina, and R. Barahona. 2018. Feeding leucaena to dairy cows and effect on milk productivity. *International Leucaena Conference 29 October – 3 November*, Brisbane 2018.

Ross, E. and Springhall, J.A. 1963. Evaluation of ferrous sulphate as a detoxifying agent for mimosine in Leucaena gluaca rations for chickens. *Australian Vet. J.* 39: 394–397.

Ruiz-González, A., S. Albores-Moreno, E. BriceñoPoot, A. Ayala-Burgos, J.Solorio-Sánchez, and J. Ku-Vera. 2011. Voluntary intake, apparent digestibility and urinary nitrogen excretion by hair sheep fed increasing levels of foliage of Leucaenu leucocephula mixed with Cynodon nlemfuezsls grass. *Advances in Animal Biosciences*. 2: 523 (Part 2)

Rushkin, F.R. 1984. *Leucaena: promising forage and tree crops for the tropics*. 2nd ed. National Research Council. Washington, DC: National Academy Press.

Schultze-Kraft, R., I.M. Rao, M. Peters, R.J. Clements, C. Bai, and G. Liu. 2018. Tropical forage legumes for environmental benefits: An overview. *Tropical Grasslands-Forrajes Tropicales* 6(1):1–14.

Shelton, M., and S. Dalzell. 2007. Production, economic and environmental benefits of leucaena pastures. *Tropical Grasslands* 41: 174–190.

Shelton, H.M. and J.L. Brewbaker. 1994. Leucaena leucocephala — the most widely used forage tree legume. In '*Forage tree legumes in tropical agriculture*.' (Eds RC Gutteridge, HM Shelton.) 15–29.

Siahaan, M.S. 1982. Lamtoro. Direktorat Jendral Peternakan, Jakarta. 22-38

Soedarjo, M., and D. Borthakur. 1996. Simple procedures to remove mimosine from young leaves, pods and seeds of Leucaena leucocephala used as food. *International Journal of Food Science + Technology*. 31(1): 97-103

Tacon, A.G. 1979. Specultive review of possible carotenoid function in fish. *Prog. Fish. Cult.* 43(4) 205-208.

Tawata, S. 1990. Effective reduction and extraction of mimosine from Leucaena and the potential for its use as a lead compound of herbicides, in: Casida J.E. (Ed.), Pesticide and Alternatives, Elsevier Science Publishers, Amsterdam. 541–544.

Tawata, S., F. Hongo, K. Sungawa, Y. Kawastima, and S. Yoga. 1986. A simple reduction method of mimosine in the tropical plant Leucaena. *Sci. Bull. College Agric. Univ. Rykyus, Okinawa*. 33: 87-93.

Ter Meulen, U., S. Struck, E, Schulke and E.A. El-Harith. 1979. A review on the nutritive value and toxic aspects of Leucaena leucocephala. *Trop Anim Prod*. 4:113-26.

Tsai, W.C. and K.H. Ling. 1971. Toxic action of mimosine − 1. Inhibition of mitosis and DNA synthesis of H.Ep-2 cell by mimosine and 3,4-dihydroxypyridine. *Toxicon* 9:241−247.

Vickery, M.L., and B. Vickery. 1981. Compound derived from amimo acids. 220- 252. in Vickery ML, Vickery B. *Secondary plant metabolism*. London.

Vijayakumar, S. and P. Srinivasan. 2018. Clinical Management of Spontaneous Leucaena leucocephala (Subabul) Poisoning in Non Descriptive Goat . *Int. J .Curr. Microbiol. App. Sci* 7(10): 1148-1151

Vogt, G., E.T. Quinito, and F.P. Pasculal. 1986. Leucaena leucocephala leaves in formulated feed for Penaeus monodon: a concerte example of the application of histology in nutrition research. *Aquaculture*. 59: 209-234.

von Son-de Fernex, E., M.A. Alonso-Diaz, P. Mendoza-de Gives, B. Valles-de la Mora, M. Gonzalez-Cortazar, A. Zamilpa, and E.G. Castillo. 2015. Elucidation of Leucaena leucocephala anthelmintic-like phytochemicals and the ultrastructural damage generated to eggs of Cooperia spp. *Vet Parasitol*. 214: 89-95.

Wee, K.L. and S.S. Wang. 1987. Nutritive value of Leucaena leaf meal in pelleted feed for Nile tilapia. *Aquaculture*. 62:97-108.

Whiting, M.G. 1982. *Food uses of Leucaena seeds. Tempeh lamtoro, preparation and toxicity*. Leucaena Research Reports 3:100-01. July. (Taipei, Taiwan: Council for Agricultural Planning and Development).

Widiyastuti, T. 2001. Detoksifikasi daun lamtoro (Leucaena leucocephala) secara fisik dan kimia serta pemanfaatannya sebagai sumber pigmentasi dalam ransum ayam broiler. tesis. Institut Pertanian Bogor.

Wiratmini, N.I., I.G.L. Oka, S. Putra, and I. G. Mahardika. 2014. The Effect of Detoxificated Leucaena leucocephala Leaf Meal to Prenatal Development of Wistar Rat Fetus. *Int. J. Pure App. Biosci.* 2 (6): 223-227

Xuan, T.D., A.A. Elzaawely, F. Deba, and S. Tawata. 2006. Mimosine in Leucaena as a potent bio-herbicide. *Agron Sustain Dev.* 26: 89–97.

Xuan, T.D., T. N. Minh, and T.D. Khanh. 2016. Isolation and biological activities of 3-hydroxy-4(1H)-pyridone, Journal of Plant Interactions, 11 (1): 94-100.

Yessirita, N., H. Abbas, Y.Heryandi, and A. Dharma. 2012. Pengaruh penggunaan kapang Trichoderma viride terhadap kandungan β-karoten pada pembiakan beberapa media tumbuh bahan pakan unggas. *Jurnal Embrio.* 5 (1): 46-53.

Zayed, M., and S. Benedict. 2016. Phytochemical constituents of the leaves of Leucaena leucocephala from malaysia. *Int J Pharm Pharm Sci*. 8:174-9.

Zayed, M.Z., M.A. Zaki, F.B. Ahmad, W.S. Ho, and S.L. Pang. 2014. Comparison Of Mimosine Content And Nutritive Values Of Neolamarckia Cadamba And Leucaena Leucocephala With Medicago Sativa As Forage Quality Index. *International Journal Of Scientific & Technology Research.* 3(8): 146-150