

## Research Paper

# A maceration treatment of leucaena foliage improves its nutritional value by reducing mimosine concentration

## *Un tratamiento de maceración del follaje de leucaena mejora su valor nutricional al reducir la concentración de mimosina*

MICHAEL D.H. HONDA<sup>1</sup>, ADEL YOUKHANA<sup>2</sup>, TRAVIS IDOL<sup>2</sup> AND DULAL BORTHAKUR<sup>1</sup>

<sup>1</sup>Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI, USA. [manoa.hawaii.edu](https://manoa.hawaii.edu)

<sup>2</sup>Department of Natural Resources and Environmental Management, University of Hawaii at Manoa, Honolulu, HI, USA. [manoa.hawaii.edu](https://manoa.hawaii.edu)

### Abstract

Giant leucaena produces high dry matter yields but the foliage contains mimosine, a non-protein amino acid that is toxic to animals, especially non-ruminants. Reducing mimosine concentration in foliage following harvesting may allow for greater use of Giant leucaena and mitigate the negative aspects of higher mimosine concentration in some varieties. We evaluated two methods for post-harvest treatment of foliage of a highly productive interspecific hybrid variety 'KX2' for reducing mimosine concentration: (i) maceration treatment; and (ii) extraction with 0.1 N HCl. Mimosine as a percentage of leaf dry matter ranged from less than 1% DM to around 3% DM. Although both methods reduced mimosine concentration, extraction by 0.1 N HCl also reduced gross energy, protein and carbohydrate concentrations of leucaena foliage. The maceration treatment, on the other hand, caused little reduction in crude protein and crude fat concentrations but markedly increased the carbohydrate concentration. ADF and NDF concentrations were also reduced as a result of maceration treatment. The estimated gross energy concentration in macerated foliage was not significantly lower than in unprocessed foliage. A suitable mechanical method for post-harvest maceration of leucaena foliage, e.g. a wood-chipping machine, could be used to reduce mimosine concentration in the foliage, making it safer for feeding to livestock and enhancing the feed value, especially for non-ruminants. These methods should be tested by conducting feeding studies to determine the possible benefits in animal performance from feeding macerated foliage.

**Keywords:** Fodder legumes, forage trees, giant leucaena, tropical forages.

### Resumen

La leucaena produce altos rendimientos de materia seca, pero el follaje contiene mimosina, un aminoácido no proteico que es tóxico para los animales, especialmente los no rumiantes. Reducir la concentración de mimosina en el follaje después de la cosecha puede permitir un mayor uso de leucaena gigante y mitigar los aspectos negativos de una mayor concentración de mimosina en algunas variedades. Evaluamos dos métodos para reducir la concentración de mimosina durante el tratamiento poscosecha del follaje de una variedad híbrida interespecífica altamente productiva 'KX2': (i) tratamiento de maceración; y (ii) extracción con 0.1 N HCl. La mimosina como porcentaje de materia seca foliar osciló entre menos del 1% y alrededor del 3% de MS. Aunque ambos métodos redujeron la concentración de mimosina, la extracción con 0.1 N HCl también redujo las concentraciones de energía bruta, proteínas y carbohidratos del follaje de leucaena. El tratamiento de maceración, por otro lado, provocó una pequeña reducción en las concentraciones de proteína cruda y grasa, pero aumentó notablemente la concentración de carbohidratos. Las concentraciones de FDA y FDN también se redujeron como resultado del tratamiento de maceración. La concentración de energía bruta estimada en el follaje macerado no fue significativamente menor que en el follaje sin procesar. Es posible usar un método mecánico adecuado para la maceración

Correspondence: Dulal Borthakur, Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI, USA. Email: [dulal@hawaii.edu](mailto:dulal@hawaii.edu)

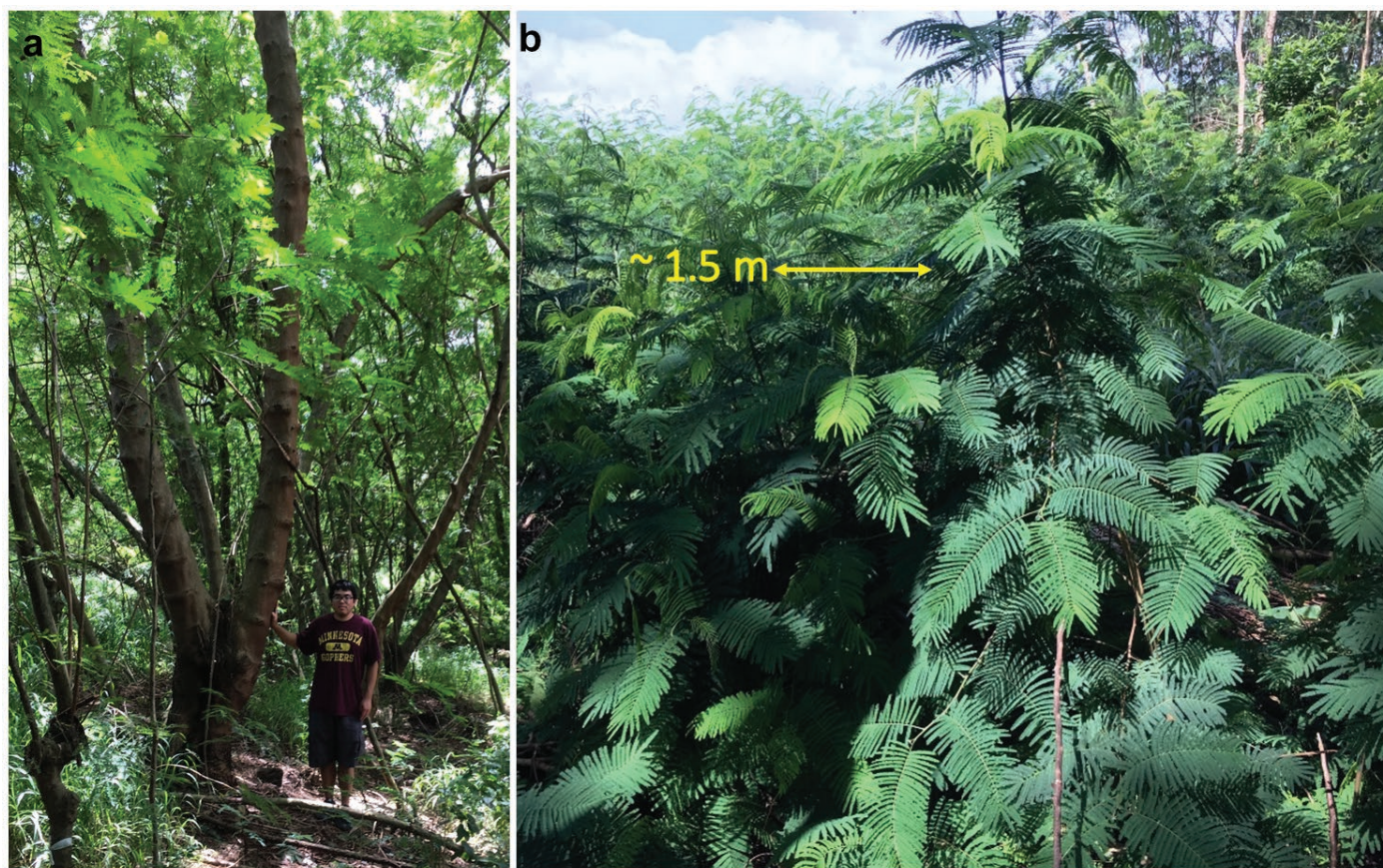
poscosecha del follaje de leucaena (p. Ej. una máquina trituradora de madera) para reducir la concentración de mimosina en el follaje, haciéndolo más seguro para la alimentación del ganado y mejorando el valor alimenticio, especialmente para los no rumiantes. Estos métodos deben probarse mediante la realización de estudios de alimentación para determinar los posibles beneficios en el rendimiento animal de la alimentación con follaje macerado.

**Palabras clave:** Árboles forrajeros, forrajes tropicales, leguminosas forrajeras, leucaena.

## Introduction

Giant leucaena (*Leucaena leucocephala* subsp. *glabrata*) is a hardy, fast-growing tree legume found in all tropical and subtropical regions of the world. It is resistant to many diseases and pests and can grow in a wide range of environmental conditions, which include drought, eroded slopes and acidic and alkaline soils (Brewbaker 2008, 2016; Honda et al. 2018). Although it normally grows as a medium-sized tree, Giant leucaena can be maintained as a bushy shrub for use as an animal fodder by repeated harvesting of its foliage during the year (Figure 1) or by pollarding through a cut-and-carry system (Youkhana and Idol 2018). Giant leucaena produces relatively fewer pods and seeds, but is still able to maintain high yielding properties. When grown as a fodder, Giant leucaena can produce as much as 99 t green forage/ha/yr (24–30 t DM/

ha/yr) (Shelton and Brewbaker 1994), which is at least 2–6 times that of Common leucaena (*Leucaena leucocephala* subsp. *leucocephala*). Since the Common type produces less biomass overall, it allocates more of the available resources to production of seeds (Table 1). Common leucaena is considered an undesirable weed due to its high seed production and potential for invasiveness (Daehler and Denslow 2019). The development of additional leucaena types, which produce fewer or no seeds but are still able to maintain high yielding properties, would be very useful. A number of Giant leucaena interspecific hybrids were developed by Dr James Brewbaker at the University of Hawaii at Manoa (Table 2) (Brewbaker 2008, 2013, 2016; Bageel et al. 2020) to improve resistance to the leucaena psyllid insect (*Heteropsylla cubana*), increase cold tolerance and/or reduce or eliminate seed production, while maintaining high productivity.



**Figure 1.** (a) Giant leucaena-KX2 for wood and timber production, and (b) Giant leucaena-KX5 bush for animal fodder.



**Table 1.** Biomass yields (t/ha/year) of Giant and Common leucaena, collected from literature. Only the top 12 yields are presented.

Type	Edible biomass (DM)	Inedible biomass (DM)	Total biomass (DM)	Edible biomass (FM)	Inedible biomass (FM)	Total biomass (FM)	References
Giant leucaena <sup>1</sup>	30.0, 31.3, 32.9, 33.3, 33.9, 34.0, 34.9, 37.1, 37.6, 38.6, 39.8, 40.3	27.2, 27.5, 28.3, 30.0, 38.8, 41.5, 73.0, 79.5, 83.0, 83.2, 93.9, 99.5	94.5, 96.5, 98.3, 100.0, 101.1, 106.8, 108.8, 110.0, 115.7, 119.4, 149.3, 152.7	62.9, 66.9, 67.7, 68.3, 68.9, 70.4, 74.2, 75.4, 93.6, 94.8, 96.0, 99.7	45.8, 46.9, 48.3, 50.7, 51.3, 53.1, 60.8, 154.3, 157.8, 163.8, 178.1, 202.0	75.8, 78.3, 78.6, 78.9, 91.6, 186.3, 194.0, 195.0, 206.5, 219.8, 253.8	Aminah and Wong (2004); Austin (1995); Austin et al. (1995); Casanova-Lugo et al. (2014); Chotchutima et al. (2016); Costa et al. (2014); López et al. (2008); Pathak and Patil (1983); Rengsirikul et al. (2011); Tudsri et al. (2019); Van den Beldt (1983)
Common leucaena	5.9, 6.0, 6.1, 6.2, 6.3, 6.5, 6.6, 6.8, 6.9, 8.5, 10.0, 10.6	9.3, 11.6, 13.8, 15.1, 17.4, 17.5, 17.9, 21.2, 23.1, 25.4, 26.8, 31.9	20.7, 22.0, 22.8, 23.1, 24.1, 24.5, 24.8, 28.1, 29.0, 33.9, 36.8, 42.5	10.4, 11.1, 14.3, 17.9, 18.1, 18.9, 18.9, 21.8, 23.3, 27.6, 32.3, 33.0	27.8, 31.9, 42.9, 43.5, 47.2, 49.7, 55.6, 58.1, 63.0, 71.1, 77.2, 86.9	38.9, 42.3, 61.0, 61.4, 61.5, 68.6, 78.9, 79.9, 81.9, 98.7, 109.5, 120.0	

<sup>1</sup>Includes K8, K636, Tarramba, Peru, Cunningham, Salvador and other types.

**Table 2.** Leucaena varieties analyzed.

Variety	Cross/parentage	Notes
Common	<i>L. leucocephala</i> subsp. <i>leucocephala</i>	Produces a lot of seeds and pods
K636	<i>L. leucocephala</i> subsp. <i>glabrata</i> (Rose) Zárte	Produces some seeds and pods
KX2	<i>L. pallida</i> x <i>L. leucocephala</i>	Self-incompatible tetraploid
KX3	<i>L. diversifolia</i> x <i>L. leucocephala</i>	Fully fertile triploid
KX4	<i>L. esculenta</i> x <i>L. leucocephala</i>	Fully sterile triploid
KX5	<i>L. diversifolia</i> x <i>L. pulverulenta</i>	Fully sterile triploid
KX7	<i>L. diversifolia</i> x <i>L. pallida</i>	Seedless hybrid

As a result of high vegetative growth and foliage production, Giant leucaena is gaining popularity as a legume fodder in many tropical and subtropical countries (Ishihara et al. 2018; Bageel et al. 2020). While it has high protein concentration and forage yields, Giant leucaena also contains high concentrations of mimosine, a toxic non-protein amino acid. Mimosine is known to have various roles in stress tolerance, such as serving as an energy storage molecule, osmolyte, phytosiderophore and antioxidant (Negi et al. 2014; Honda and Borthakur 2019, 2020, 2021; Rodrigues-Corrêa et al. 2019). Mimosine binds with Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and pyridoxal-5' phosphate (PLP) (Negi et al. 2013, 2014), which are important cofactors for many enzymes involved in various biochemical pathways. A disruption of these pathways by mimosine leads to toxic side effects that include goiter, thyroid problems, fetal defects, infertility and hair loss (Crounse et al. 1962; Hamilton et al. 1968; Joshi 1968; Dewreede and Wayman 1970). Although mimosine is present in all parts of the leucaena plant, its concentrations are highest in the growing shoot tips (14–22% DM) and seeds (~6% DM) (Soedarjo and Borthakur 1996; Honda and Borthakur 2019).

Some bacteria, such as *Rhizobium* strain TAL1145, which forms nitrogen-fixing root nodules on leucaena, and certain rumen bacteria such as *Synergistes jonesii*, have

abilities to degrade and detoxify mimosine (Allison et al. 1992; Soedarjo et al. 1994). Mimosinase, an enzyme present in the leucaena chloroplasts, also degrades mimosine under certain stress environments, such as high heat (Negi et al. 2014). The complete degradation of mimosine by mimosinase produces pyruvate, ammonia and 3-hydroxy-4-pyridone (3H4P), which is further degraded by a dioxygenase enzyme to pyruvate, formate and ammonia (Awaya et al. 2005, 2007; Negi et al. 2014; Negi and Borthakur 2016). The mimosine-degradation product 3H4P, its tautomer 3,4-dihydroxypyridine (3,4DHP) and its isomer 2,3-dihydroxypyridine (2,3DHP) can also cause toxic side effects in animals that include reduced feed intake, goiter and kidney and liver problems (Hegarty et al. 1979). This toxicity limits the use and acceptability of leucaena as an animal fodder, especially in non-ruminants. The toxic effects of mimosine and 2,3DHP can be countered through animal inoculation with *Synergistes jonesii* (Jones 1981). However, in a study conducted by Haliday et al. (2018), it was found that inocula of *S. jonesii* did not fully protect *Bos indicus* steers from 2,3DHP toxicity in Queensland, Australia. Leucaena toxicity, as indicated by high DHP levels, is still common in tropical countries that feed leucaena to ruminants (Haliday et al. 2013). Dalzell et al. (2012) found that almost

50% of herds in Queensland, Australia, including those previously inoculated, were unprotected from mimosine and DHP toxicity. In that study, the authors concluded that 3,4DHP and 2,3DHP toxicity remained a problem and was likely limiting animal production in some leucaena pastures. However, Shelton et al. (2019) postulated that inoculation with rumen bacteria may not be necessary for certain cattle populations. They observed that 2,3DHP was excreted in the urine of Bali bulls as a glycosylated conjugate. Degradation by rumen bacteria or excretion in the urine, both help to detoxify the effects of mimosine in leucaena foliage; however, a significant amount of energy is wasted when mimosine is excreted in urine, since glycosylation of xenobiotic compounds by UDP-sugars requires glucose and ATPs.

One possible way to combat mimosine and 2,3DHP toxicity would be to remove mimosine through post-harvest processing and two methods of doing so have been mentioned in the literature. Soedarjo and Borthakur (1996) developed a simple soaking method that removed up to 97% of mimosine from young leaves, pods and seeds. Recently, Honda and Borthakur (2019) found that maceration and incubation of leucaena leaflets in an alkaline buffer solution significantly reduced their mimosine concentration. Mimosinase was found to be present in greater concentrations in leucaena leaves than in roots (Honda et al. 2019). While mimosine and mimosinase are both present in leucaena foliage, they are spatially separated under normal growth conditions (Negi et al. 2014). However, mimosinase is released from broken chloroplasts when leaves are macerated and come in contact with mimosine, and consequently mimosine is degraded. Mimosinase is a relatively stable and efficient enzyme that remains active for several hours at room temperature (Negi et al. 2014).

We considered that it would be possible to develop a processing method to lower mimosine levels in harvested leucaena foliage. Accordingly, we tested two methods of processing leucaena forage, including maceration of leucaena leaves, to reduce mimosine in foliage and hence reduce toxicity, especially for non-ruminants.

## Materials and Methods

### *Sampling location*

Leaf samples of Common leucaena and Giant leucaena hybrid varieties K636, KX2, KX3, KX4, KX5 and KX7 were collected from the Waimanalo research station, University of Hawaii, Waimanalo, HI.

### *Mimosine extraction and quantification*

Mimosine and 3H4P were extracted from leaves of these varieties following the methods described by Honda and Borthakur (2019) and their concentrations were calculated.

### *Crude protein extraction and quantification*

Crude protein was extracted from leucaena green foliage following the methods described by Tsugama et al. (2011). Nitrogen was quantified using the Bradford assay and using bovine serum albumin (BSA) as the standard. Each sample set contained six replicates.

### *Dry matter concentrations in Common leucaena and various Giant leucaena varieties*

Water and dry matter concentrations in leaves were determined gravimetrically. Crude protein was extracted from leucaena green foliage following the methods described by Tsugama et al. (2011). Nitrogen was quantified using the Bradford assay and using BSA as the standard. Each sample set contained six replicates.

### *Above-ground biomass yields of KX2 trees*

Leucaena variety KX2 was selected for mimosine reduction experiments because it is a cultivar with high mimosine concentration, and it is readily available for sample collection and analyses. KX2 has also been previously tested and registered (Brewbaker 2008, 2016; Youkhana and Idol 2009, 2016). Above-ground biomass growing from the stumps of 3-year-old trees was determined following the methods described by Youkhana and Idol (2011).

### *Processing methods to reduce mimosine in KX2 leaves*

Two processing methods were tested: (a) In the maceration method, 1 g of fresh leaves was macerated for 1 min using a mortar and pestle with no added water or solvent. Following maceration, the ground leaves were transferred to a petri dish and allowed to incubate at 25 °C overnight in the dark. It was expected that maceration would release mimosinase from leaves and incubation would induce mimosine degradation by the mimosinase (Negi et al. 2014). After incubation, macerated leaves were dried for 24 h at 65 °C. (b) In the acid treatment method, 1 g of fresh leucaena leaves was submerged in 30 mL of 0.1 N HCl.

Samples were shaken vigorously for 1 min and then shaken moderately overnight at room temperature. After shaking, the acid extracts were decanted and the leaves rinsed several times with distilled H<sub>2</sub>O before drying in a baking oven for 24 h at 65 °C. Fresh leaves were dried for 24 h at 65 °C to serve as unprocessed Controls. After drying, processed and unprocessed (control) leaves were ground into a fine powder using a mortar and pestle. Mimosine and 3H4P were extracted by placing 200 mg of dried, ground leucaena leaves and 30 mL of 0.1 N HCl in a 50 mL conical tube. Mimosine and 3H4P concentrations were quantified following the methods described above. Six replicate leaf samples were processed using each method.

*Gross energy concentration in unprocessed (Control) and processed (macerated) KX2 leaves*

Dried, ground leucaena leaves were sent to the Wildlife Habitat and Nutrition Lab in the School of the Environment, Washington State University, Pullman, WA for determination of gross energy (GE) concentration using a bomb calorimeter. Twelve replicates of each treatment were analyzed.

*Nutrient profile of unprocessed (Control) and processed (macerated) KX2 leaves*

To study the effects of maceration on the nutrient concentration in leucaena leaves, protein, crude fat, carbohydrate, ADF and NDF concentrations were determined for dried, ground macerated and unprocessed (control) leaves.

Crude protein extracts were collected and nitrogen quantified following the methods described above. Each sample set contained six replicates.

Dried, ground leucaena leaves were sent to the Agricultural Diagnostic Services Center (ADSC), CTAHR, University of Hawaii at Manoa for determination of crude fat by the ether extract method. Each sample set contained six replicates.

Carbohydrates were extracted from leucaena leaves and quantified following the methods described by Robbins and Pharr (1988) and Yemm and Willis (1954), using dextrose as the standard. Each sample set contained six replicates.

Dried, ground leucaena leaves were sent to Wildlife Habitat and Nutrition Lab in the School of the Environment, Washington State University, Pullman, WA for determination of ADF and NDF concentrations. Each sample set contained six replicates.

To balance the GE stoichiometry of unprocessed (Control) and macerated leucaena leaves, the kcals of proteins, fats and carbohydrates were assumed to be 4, 9 and 4 kcal/g, respectively. In a study conducted by Kienzle et al. (2001), it was found that the heat combustion of cellulose and lignin were found to be approximately 17.5 kJ/g and 25.5 kJ/g, respectively, which, when converted to kcals, were 4.2 kcal/g and 6.1 kcal/g, respectively. Therefore, for this study, ADF and NDF are assumed to have gross energy concentrations of 5.0 kcal/g each.

*Determination of proanthocyanidin concentrations in unprocessed and processed KX2 leaves*

Proanthocyanidins (PAs) were extracted from leucaena leaves using 70% acetone and quantified from the extracts using the butanol-HCl assay previously utilized by Dalzell and Kerven (1998) and Shay et al. (2017). Epigallocatechin was used as the standard. Each sample set contained six replicates.

*Determination of total phenol concentration in unprocessed and processed KX2 leaves*

Total phenols (TP) were extracted from leucaena leaves using 70% acetone and were quantified using the Folin Ciocalteu method (Zarin et al. 2016). Each sample set contained six replicates.

*DPPH assay of unprocessed and processed KX2 leaf extracts*

The radical scavenging capabilities of leucaena leaves were determined using the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) assay (Mishra et al. 2012). Ascorbic acid was used as the control. Each sample set contained six replicates.

*Statistical analysis*

For all parameters measured, a Student's t-test for variance was used to determine statistical significance at P<0.05.

## Results

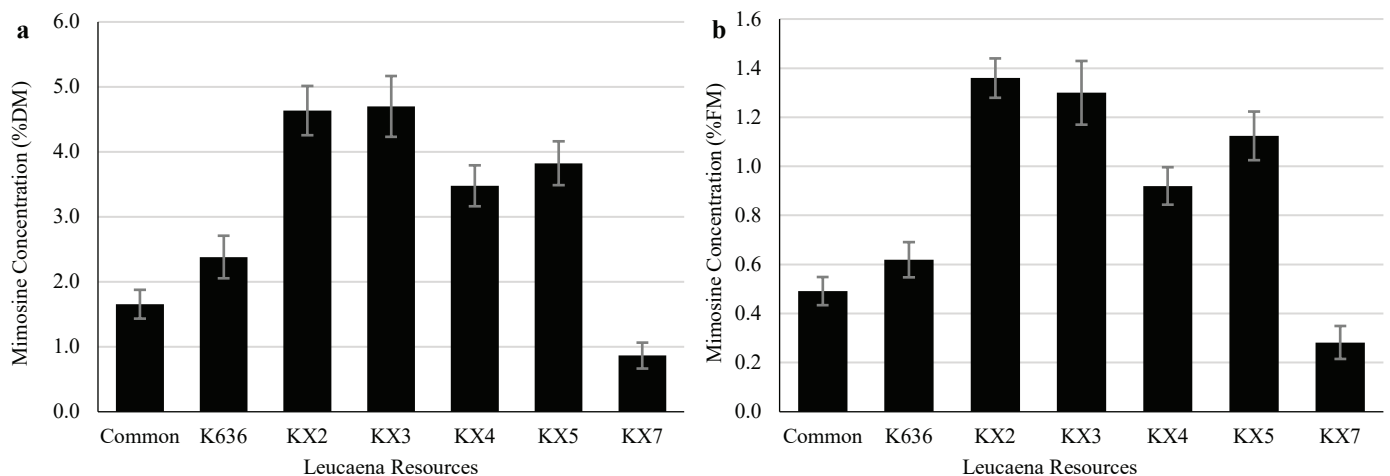
*Mimosine and dry matter concentrations*

Among the various leucaena types tested, Giant leucaena

KX7 had the lowest leaf mimosine concentration (0.87% DM), followed by Common leucaena (1.65% DM) and Giant leucaena K636 (2.38% DM) (Figure 2a). Leucaena hybrids KX2 and KX3 had the highest leaf mimosine concentrations (4.6–4.7% DM). On the basis of fresh matter (FM), leaf mimosine concentrations of Common leucaena and the various Giant leucaena hybrids ranged from 0.28 to 1.36% FM (Figure 2b). The dry matter content of leaves for different leucaena hybrids ranged from ~ 26–30% DM (Table 3). The protein content of leaves for different leucaena hybrids ranged from ~ 11–17% DM.

#### Protein concentration

The protein concentration in green foliage was determined for the various leucaena varieties. The entire green foliage including soft green stems is generally foraged upon by browsers and tip leaves are usually young and immature relative to other leaf types. These leaves generally contained more protein than middle and base leaves, which are usually older and more mature than tip leaves and had similar protein concentrations (Figure 3). These results indicated that a large amount of protein is contained in the young and immature parts of leucaena foliage. Interestingly, green stems had protein concentrations similar to those of middle and base leaves, which indicated that green stems were also a good source of protein. Protein concentrations in the entire young branches (leaves and green stem) of the various leucaena types tested ranged from 3.0 to 5.2% FM. For the most part, the combined green foliage of Giant leucaena varieties contained more protein than the combined green foliage of Common leucaena.



**Figure 2.** Mimosine concentration as % of (a) dry matter and (b) fresh matter of the leaves of Giant leucaena ‘KX7’, common leucaena, Giant leucaena ‘K636’, ‘KX2’, ‘KX3’, KX4’ and ‘KX5’. Error bars indicate standard error of six replicates.

#### Above-ground biomass production

The above-ground biomass production from regrowth of 3-year-old leucaena KX2 trees was found to be 29.7 kg DM/tree (Figure 4). Stems contributed almost 64% of the total biomass of these trees.

**Table 3.** Dry matter and crude protein concentrations ( $\pm$  s.e.) in leaves of Common leucaena and Giant leucaena varieties.

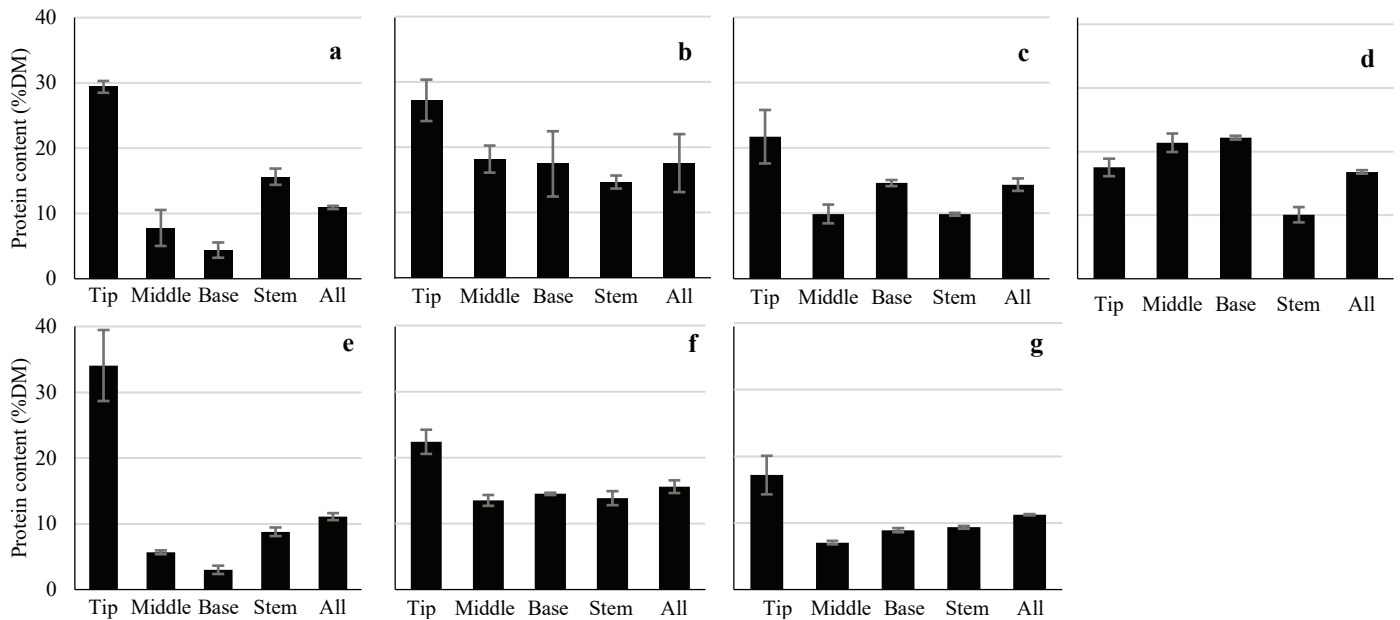
Variety	Dry matter (%)	Crude protein (%DM)
Common leucaena	30.2 $\pm$ 0.5	10.9 $\pm$ 0.3
Giant leucaena K636	29.9 $\pm$ 1.2	17.6 $\pm$ 4.4
Giant leucaena KX2	26.8 $\pm$ 0.8	14.4 $\pm$ 1.0
Giant leucaena KX3	29.4 $\pm$ 0.6	16.8 $\pm$ 0.3
Giant leucaena KX4	26.7 $\pm$ 1.2	11.1 $\pm$ 0.5
Giant leucaena KX5	27.8 $\pm$ 0.8	15.6 $\pm$ 1.0
Giant leucaena KX7	32.1 $\pm$ 0.3	11.2 $\pm$ 0.9
All	28.9 $\pm$ 0.4	13.9 $\pm$ 2.9

#### Mimosine and 3H4P concentrations in leaves

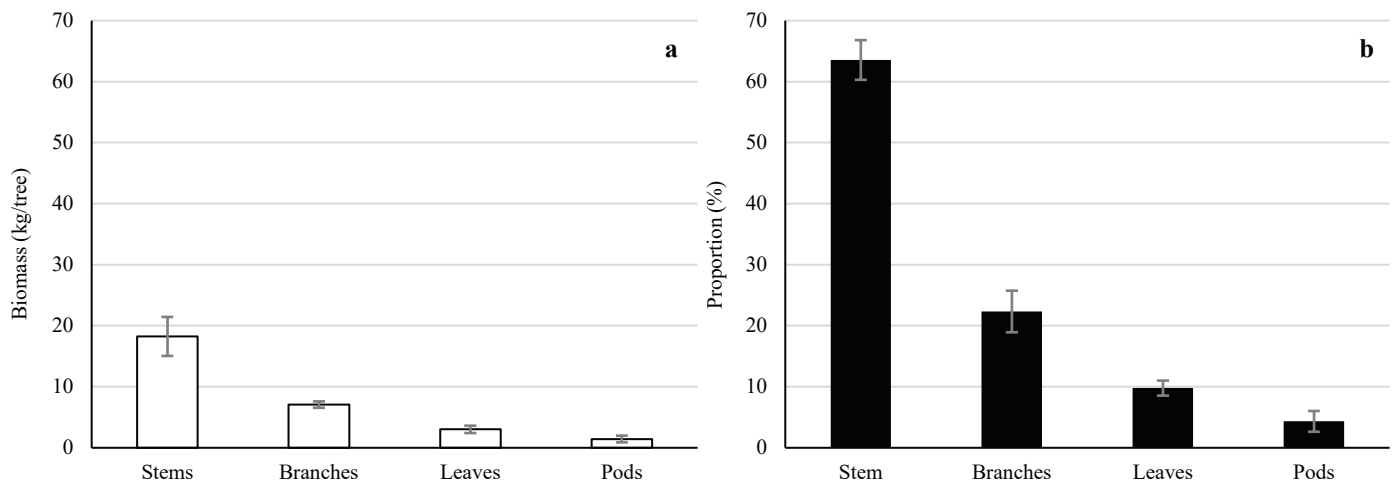
Both maceration and treatment with 0.1 N HCl significantly reduced mimosine concentrations in leucaena leaves (Figure 5a). The maceration treatment slightly increased 3H4P concentration, while treatment with 0.1 N HCl significantly reduced 3H4P in leaves.

#### Gross energy concentrations in KX2 leaves

Unprocessed leaves had a gross energy (GE) concentration of 4,708 cal/g DM (Figure 5b), while macerated leaves had a GE concentration of 4,715 cal/g DM ( $P > 0.05$ ). On the other hand, leaves processed using the 0.1 N HCl method had a GE concentration of 3,454 cal/g DM, which is more than 25% lower than unprocessed Controls. These



**Figure 3.** Crude protein contents (%DM) of tip leaves, middle leaves, base leaves, green stems <5 mm in diameter, and all parts of the foliage (leaves and green stems) for (a) Common leucaena, (b) Giant leucaena 'K636', (c) Giant leucaena 'KX2', (d) Giant leucaena 'KX3', (e) Giant leucaena 'KX4', (f) Giant leucaena 'KX5', (g) Giant leucaena 'KX7'. Errors bars indicate standard error of six replicates.



**Figure 4.** (a) The above ground biomass (kg/tree) of stem, branches, leaves and pods of 3-year-old Giant leucaena 'KX2', 6 months after pollarding; and, (b) the proportion (%) that stem, branches, leaves, and pod contribute to the total aboveground biomass. Error bars indicate standard error of seven replicates.

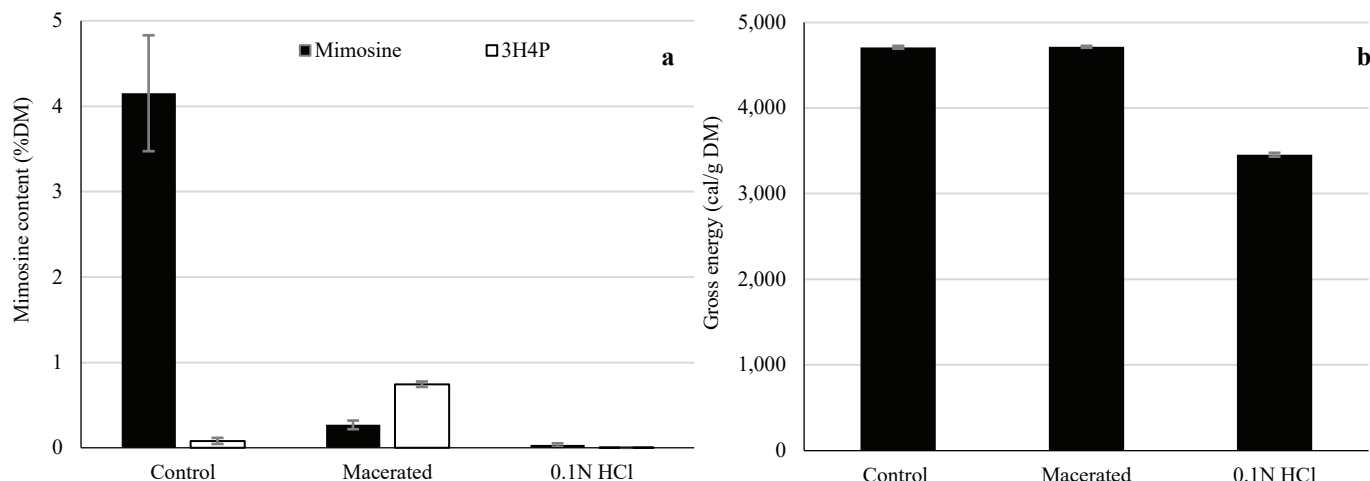
results indicate that a large amount of energy is lost when leucaena leaves are processed using 0.1 N HCl.

#### Macronutrient concentrations

Since processing leucaena leaves can reduce mimosine levels in the foliage, it is possible that processing can also affect important nutrients as well. Extracts of leucaena leaves processed with 0.1 N HCl were found to contain both proteins and carbohydrates, indicating that both macronutrients were removed along with mimosine (data not shown). Therefore, the nutrient profile of

leaves processed by 0.1 N HCl was not determined as it significantly reduced nutritional value by lowering protein, carbohydrate and gross energy concentrations. Maceration of leucaena leaves significantly reduced the mimosine concentration, but did not affect the GE concentration, so the nutrient profile was determined for macerated leucaena leaves and compared with unprocessed Control leaves. The protein concentration in macerated leucaena leaves was found to be 17.0% (DM basis), which was slightly lower than for the unprocessed Control leaves (18.5%) (Figure 6a). Macerated leaves also had a lower crude fat concentration (3.8% DM) than the





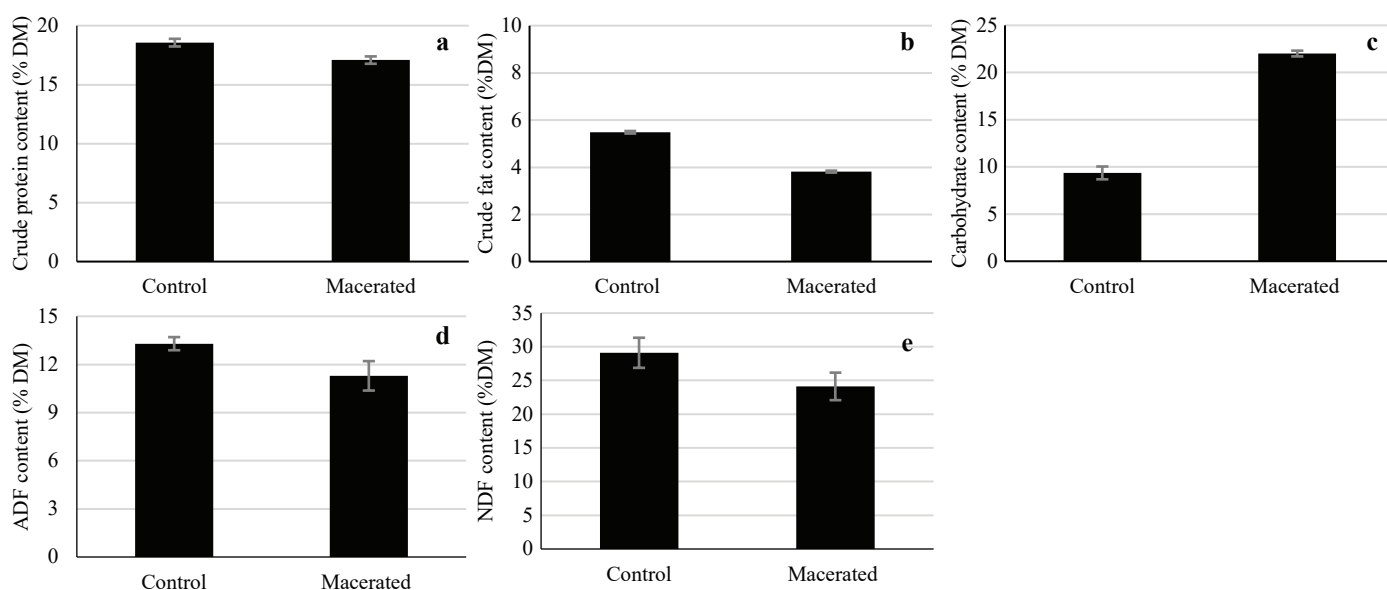
**Figure 5.** (a) Left-over mimosine and 3H4P, and (b) gross energy contents of Giant leucaena ‘KX2’ leaves after processing to remove mimosine. Processing methods included maceration and extraction with 0.1N HCl. Unprocessed leaves served as the control. Error bars indicate standard error of twelve replicates.

unprocessed Control leaves (5.5% DM), suggesting that some degradation of lipids occurred during maceration (Figure 6b). Interestingly, macerated leaves had a much higher carbohydrate concentration (22.0% DM) than unprocessed Control leaves (9.4% DM) (Figure 6c). Both ADF and NDF concentrations in macerated leaves were found to be significantly lower than in unprocessed Control leaves (Figures 6d and 6e). The increases in carbohydrate concentration were, therefore, related to decreases in mimosine, protein, crude fat, ADF and NDF concentrations of macerated leucaena leaves. When gross energy concentration of the macerated leucaena leaves was calculated on the basis of these macronutrients, the gross energy estimate was found to

be slightly lower than that for the unprocessed Control, but differences were not significant ( $P = 0.584$ ) (Table 4).

#### *Total phenol, proanthocyanidin and DPPH radical scavenging assay of KX2 leaves following mimosine reduction treatment*

The proanthocyanidin (PA) concentration in macerated leucaena foliage was significantly lower than in unprocessed Control leaves, suggesting that some condensed tannins were degraded during maceration (Figure 7a). However, there was no significant difference in the total phenolic concentrations between macerated leaves and unprocessed Control leaves (Figure 7b).



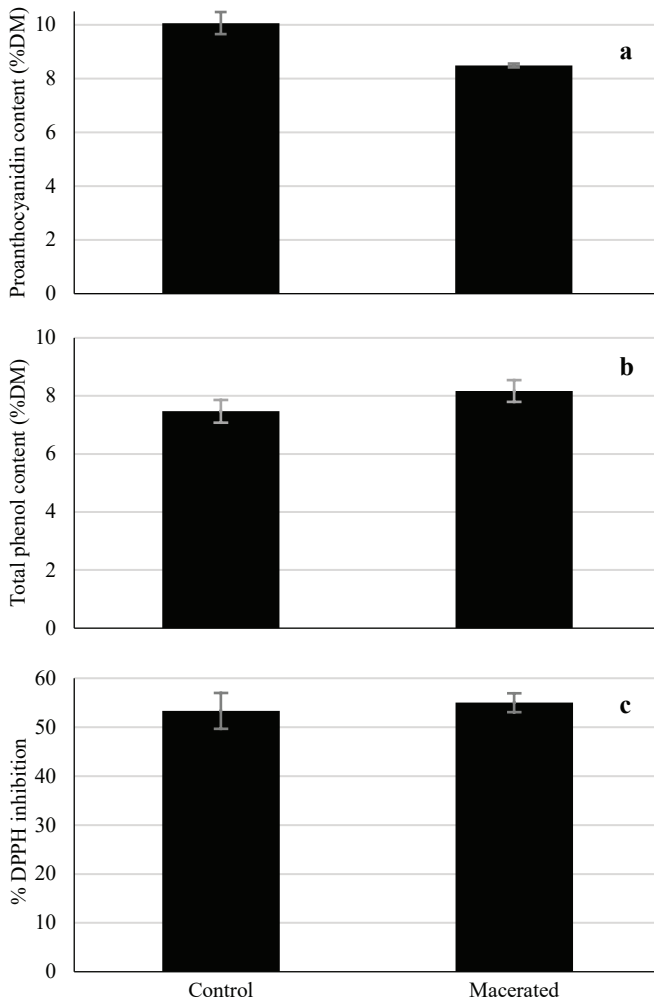
**Figure 6.** (a) Crude protein, (b) crude fat, (c) carbohydrate, (d) ADF and (e) NDF contents of control (unprocessed) and macerated (processed) Giant leucaena ‘KX2’ leaves. Error bars indicate standard error of six replicates.



Similarly, the DPPH radical scavenging activities were not significantly different between macerated leaves and unprocessed leaves (Figure 7c).

**Table 4.** Calculated gross energy concentrations in macerated and unprocessed (control) leaves of Giant leucaena 'KX2'. Energy in proteins and carbohydrates is assumed to be 4 kcal/g DM ( $\pm$  s.e.), fat 9 kcal/g and ADF and NDF 5 kcal/g.

	Calculated gross energy concentration (total kcal/kg DM)	
	Unprocessed Control	Macerated
Protein	743 $\pm$ 14	684 $\pm$ 12
Fat	494 $\pm$ 05	342 $\pm$ 03
Carbohydrate	376 $\pm$ 01	880 $\pm$ 03
ADF	665 $\pm$ 08	565 $\pm$ 19
NDF	1,455 $\pm$ 46	1,205 $\pm$ 42
Total	3,733 $\pm$ 55	3,676 $\pm$ 80



**Figure 7.** (a) Proanthocyanidin contents (epicatechine equivalent), (b) total phenol contents (tannic acid equivalent), and (c) DPPH radical scavenging properties of control (unprocessed) and macerated (processed) Giant leucaena 'KX2' leaf extracts. Error bars indicate standard error of six replicates.

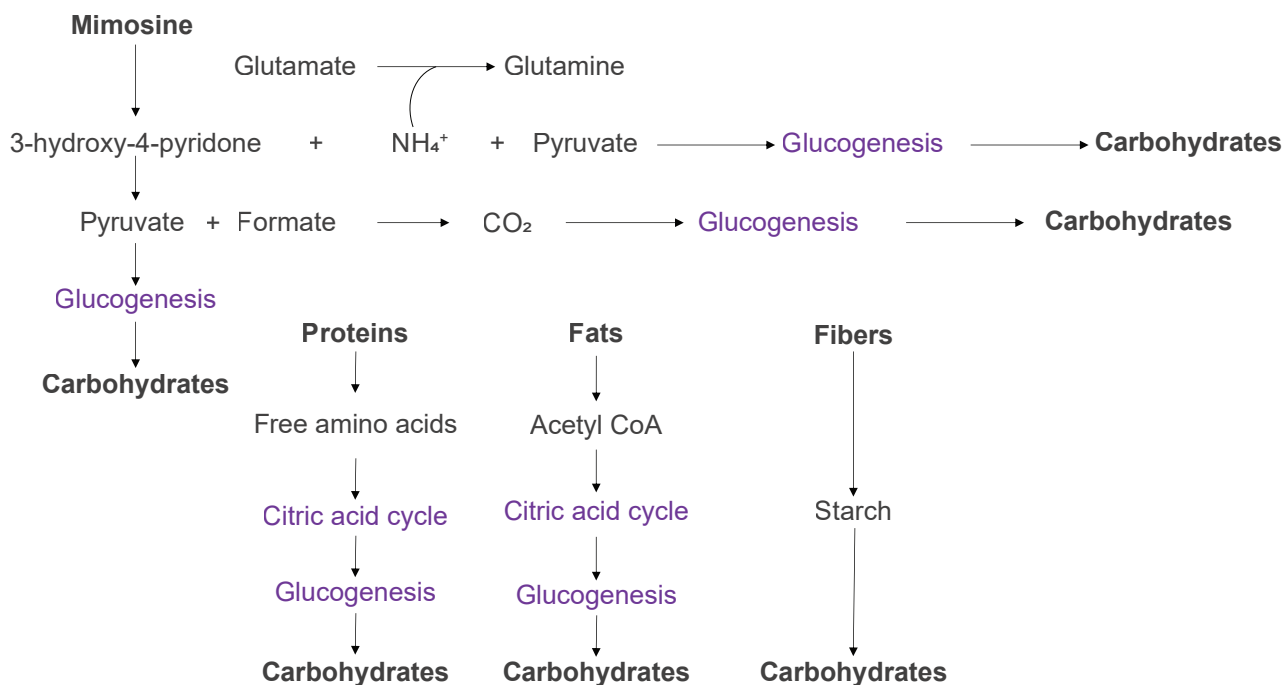
## Discussion

In this study, the estimated mean protein concentration of the edible biomass (leaves and green stems) of all Giant leucaena types tested was 139 g/kg DM. Thus, with a green forage yield of 63–100 t/ha/year (Table 1), Giant leucaena can produce 2,579–4,088 kg protein/ha/year, which is much higher than the protein yields of alfalfa (*Medicago sativa*) (Brewbaker et al. 1972; ter Meulen et al. 1979). In addition to being a high protein producer, Giant leucaena is considered an ideal fodder legume for the tropics for a number of other reasons: (i) it can be grown at high plant density of 20,000 plants/ha (Van den Beldt and Brewbaker 1980); (ii) it grows well in marginal lands, dry areas and eroded slopes; (iii) as a nitrogen-fixing tree legume it fixes high amounts of N (196–268 kg N/ha) in nodule-forming symbiosis with *Rhizobium* (Sanginga et al. 1989); (iv) because of its deep root system and drought tolerance, it can be grown as a rain-fed fodder without irrigation; and (v) as a perennial fodder, it does not require annual replanting and can be maintained with minimum effort and resources. However, despite these desirable attributes, Giant leucaena is often misunderstood to be the same as its close relative 'Common leucaena', which is considered to be an invasive weed (Daehler and Denslow 2019). Giant leucaena is generally much less invasive than Common leucaena and is grown in various countries throughout the world such as Thailand, Indonesia and Colombia, where it is used as nutritious animal fodder. In addition, a number of self-sterile, fully sterile and low seed-producing hybrid varieties, developed by Dr James Brewbaker, are currently available for cultivation (Brewbaker 2008, 2013, 2016; Bageel et al. 2020). Leucaena hybrid varieties with reduced mimosine concentrations would increase fodder value for feeding, especially to non-ruminants. Mimosine concentrations in Common leucaena and some Giant leucaena hybrid varieties ranged from 0.8 to 4.7% DM with Common leucaena and Giant leucaena variety KX7 having the lowest mimosine concentrations among all varieties tested. Unfortunately, KX7 is a seedless hybrid that has low productivity and is therefore unsuitable for fodder use (unpublished results). Similarly, Common leucaena is unsuitable for fodder use due to its high seed production and invasiveness. While Giant leucaena variety KX2 had high biomass production (Mullen and Gutteridge 2002), it had one of the highest mimosine concentrations of all varieties tested in this study. In a field experiment conducted in Hawaii by Youkhana and

Idol (2016), KX2 plants were pollarded every 6 months and total production was 65 t mulch DM/ha over 3 years. Currently, there are no other data available on long-term sustainable production of KX2 harvested regularly for use as forage for stock.

Although processing of leaves using 0.1 N HCl was highly effective at reducing mimosine concentrations in foliage, significant amounts of gross energy and macronutrients were also lost in the extraction process. On the other hand, maceration treatment of leucaena leaves reduced mimosine concentration in the foliage by >93% without causing any loss in gross energy. During maceration of leucaena foliage, mimosinase, enzymes for  $\beta$ -oxidation and various proteases and cellulases are released from chloroplasts, mitochondria, peroxisomes and other subcellular compartments (Lowry et al. 1983; Honda and Borthakur 2019). Mimosinase in leucaena tissues degrades mimosine into 3H4P, pyruvate and ammonia (Negi et al. 2013; Negi and Borthakur 2016). 3H4P can be further degraded to pyruvate, formate and ammonia (Awaya et al. 2005). The two pyruvate molecules formed may be converted to acetyl-CoA by pyruvate dehydrogenase complexes, which may be released from the breakdown of plastids and mitochondria. The two ammonia molecules produced may be converted to glutamine by glutamine synthetases present in the plant cytoplasm and chloroplasts. Chloroplastic glutamine

synthetase was shown to be a stable enzyme that remained active at 30 °C for >1 h (Ericson 1985). The enzymes for  $\beta$ -oxidation may convert a portion of lipids and fatty acids into acetyl-CoA. Proteases may convert proteins into smaller peptides and amino acid chains; and similarly, cellulases may partially degrade large ADF and NDF fibers into simple carbohydrates (Hayashi et al. 2004). A leucaena transcriptome analysis revealed the presence of a number of cellulose- and hemicellulose-degrading enzymes that were shown to be expressed in the roots and shoots of Giant leucaena (Honda et al. 2019). Forages that have low ADF have higher digestible energy than forages with high ADF, and excess NDF concentration in animal forage limits feed intake (Mertens 1987; Obregón-Cano et al. 2019). Crude fat, crude protein, ADF and NDF concentrations were also reduced by processing through maceration, which may have led to the significant increase in carbohydrate concentration. The calculated gross energy concentrations in macerated and unprocessed Control leaves were not significantly different, indicating that the loss of gross energy in macerated leaves through degradation of some protein, fat, ADF and NDF has been balanced by increases in carbohydrates. The possible pathways for carbohydrate synthesis from the degradation products of mimosine, protein, lipids, ADF and NDF in macerated leucaena tissues, are shown in Figure 8.



**Figure 8.** Predicted biochemical pathways in macerated leucaena foliage that lead to the increase in carbohydrate content, resulting from the decreases in mimosine, protein, fat and fiber contents.

Although it has been shown that DHP derived from mimosine can be excreted in animal urine as a glycosylated conjugate (Shelton et al. 2019), a sizable amount of energy is lost when mimosine is removed or not utilized by animals. To remove one molecule of DHP in the urine, it must be conjugated to a glucuronic acid (GA) molecule by UDP-GA, derived from UDP-glucose (Meng et al. 2019; Shelton et al. 2019). That means for every one molecule of mimosine consumed, one molecule of ATP (UTP equivalent) and one molecule of glucose are used. To put things in perspective, if a cow consumes 10 kg DM/day of leucaena foliage, containing 30 g mimosine/kg DM (3% DM), it will require 300 g of mimosine to be metabolized and excreted per day. To do this, the molar equivalent of 300 g of mimosine in the form of glucose and ATP must be diverted from normal metabolism to generation of UDP-GA. Metabolism and excretion of mimosine and its degradation products are energetically wasteful, especially if large amounts of mimosine are present in leucaena foliage. Besides costing energy to remove mimosine, additional energy is lost since mimosine is not utilized for energy by animals. Complete degradation of mimosine and 3H4P produces two molecules of pyruvate, the same amount as one glucose molecule produces in glycolysis. In addition, mimosine (MW=198.18) contains eight carbon, two nitrogen, four oxygen and ten hydrogen atoms, which is stoichiometrically equivalent to 0.67 glucose ( $C_6H_{12}O_6$ ; MW = 180.2) molecules. That means three molecules of mimosine contain the same amount of carbon, oxygen and hydrogen atoms as at least two glucose molecules, with extra carbon and nitrogen atoms to spare. This means that if the concentration of mimosine within leucaena foliage is 30 g mimosine/kg DM, and if cattle consume leucaena foliage in the amount of 10 kg DM/day, then theoretically the stoichiometric equivalent of 200 g of glucose is lost in a day. Post-harvest maceration of leucaena foliage reduces mimosine concentration significantly and increases carbohydrate concentration. Therefore, consumption of non-macerated foliage will cost some energy in the form of glucose; however, consumption of macerated foliage will add energy in the form of carbohydrates.

Post-harvest maceration of leucaena foliage seems a useful and efficient processing method for large-scale harvests of Giant leucaena varieties that contain high mimosine concentrations. The use of wood-chipping machinery is a possible method to macerate leucaena foliage. This method may be useful in cut-and-carry systems, which are widely used in ruminant feeding in Indonesia (Panjaitan et al. 2010). According to Shelton et al. (2019), Indonesian cattle naïve to leucaena overcome toxicity

symptoms within a relatively short period and produce excellent growth performance. Although ruminants are able to combat mimosine and 2,3DHP/3,4DHP toxicity through inoculation with ruminant bacteria or through glucuronidation and excretion in urine, animal performance may be enhanced through post-harvest maceration of leucaena tissue. Besides reducing mimosine levels, maceration treatment also significantly reduces the proanthocyanidin (PA) concentration in leucaena foliage. PAs can bind polysaccharides and proteins to form insoluble complexes, which affect digestion and absorption of these macronutrients (Zhong et al. 2018; Reed 2001). In addition, a sizable amount of energy and resources that normally would have been used to remove mimosine from animals will not be wasted, and the energy stored in the form of mimosine will be converted into usable forms. Macerating leucaena foliage should increase fodder value of the forage by: (i) reducing components that inhibit nutrient absorption, such as mimosine, ADF, NDF and proanthocyanidins; (ii) increasing the amount of bioavailable macronutrients, i.e. carbohydrates; and (iii) performing a role similar to pre-masticating of the leucaena foliage by ruminants, helping them in feed digestion and nutrient absorption.

## Conclusion

While acid treatment of leucaena forage reduced mimosine, protein, carbohydrate and gross energy levels in the forage, maceration was also successful in reducing mimosine concentration while having little effect on gross energy levels by increasing carbohydrate concentration. Maceration could be useful for treating forage of Giant leucaena hybrids that have high yields but relatively high mimosine concentrations, such as K636, KX2, KX3, KX4 and KX5. Larger-scale production of macerated foliage could be accomplished by using a wood-chipping machine. This strategy should be tested by conducting feeding studies with both ruminants and non-ruminants and, if successful, could be used in a 'cut-crush-and-carry' system for feeding farm animals.

## Conflict of interest

The authors declare they have no conflict of interest.

## Acknowledgments

This work was supported by a Hatch grant from the USDA National Institute of Food and Agriculture, managed by the College of Tropical Agriculture and Human Resources.



## References

(Note of the editors: All hyperlinks were verified 9 December 2021).

- Allison MJ; Mayberry WR; McSweeney CS; Stahl DA. 1992. *Synergistes jonesii*, gen. nov., sp. nov.: a rumen bacterium that degrades toxic pyridinediols. Systematic and Applied Microbiology 15:522–529. doi: [10.1016/S0723-2020\(11\)80111-6](https://doi.org/10.1016/S0723-2020(11)80111-6)
- Aminah A; Wong CC. 2004. Dry matter productivity and nutritive quality of leucaena hybrid lines for high protein feed production. Journal of Tropical Agriculture and Food Science 32:251–256. [citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.1069.1298](https://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.1069.1298)
- Austin MT. 1995. Agronomic potential of leucaena species and hybrids in Hawaii. Ph.D. Thesis. University of Hawaii, Honolulu, HI, USA. [hdl.handle.net/10125/56268](https://hdl.handle.net/10125/56268)
- Austin MT; Sorensson CT; Brewbaker JL; Sun WG; Shelton HM. 1995. Forage dry matter yields and psyllid resistance of thirty-one leucaena selections in Hawaii. Agroforestry Systems 31:211–222. doi: [10.1007/BF00712074](https://doi.org/10.1007/BF00712074)
- Awaya JD; Fox PM; Borthakur D. 2005. *pyd* genes of *Rhizobium* sp. strain TAL1145 are required for degradation of 3-hydroxy-4-pyridone, an aromatic intermediate in mimosine metabolism. Journal of Bacteriology 187:4480–4487. doi: [10.1128/JB.187.13.4480-4487.2005](https://doi.org/10.1128/JB.187.13.4480-4487.2005)
- Awaya JD; Walton C; Borthakur D. 2007. The *pydA-pydB* fusion gene produces an active dioxygenase-hydrolase that degrades 3-hydroxy-4-pyridone, an intermediate of mimosine metabolism. Applied Microbiology and Biotechnology 75:583–588. doi: [10.1007/s00253-007-0858-3](https://doi.org/10.1007/s00253-007-0858-3)
- Bageel A; Honda MDH; Carillo JT; Borthakur D. 2020. Giant leucaena (*Leucaena leucocephala* subsp. *glabrata*): a versatile tree-legume for sustainable agroforestry. Agroforestry Systems 94:251–268. doi: [10.1007/s10457-019-00392-6](https://doi.org/10.1007/s10457-019-00392-6)
- Brewbaker JL. 2008. Registration of ‘KX2-Hawaii’, interspecific-hybrid leucaena. Journal of Plant Registrations 2:190–193. doi: [10.3198/jpr2007.05.0298crc](https://doi.org/10.3198/jpr2007.05.0298crc)
- Brewbaker JL. 2013. ‘KX4-Hawaii’, seedless interspecific hybrid leucaena. HortScience 48:390–391. doi: [10.21273/HORTSCI.48.3.390](https://doi.org/10.21273/HORTSCI.48.3.390)
- Brewbaker JL. 2016. Breeding leucaena: Tropical multipurpose leguminous tree. Plant Breeding Reviews 40:43–121. doi: [10.1002/9781119279723.ch2](https://doi.org/10.1002/9781119279723.ch2)
- Brewbaker JL; Plucknett DL; Gonzalez V. 1972. Varietal variation and yield trials of *Leucaena leucocephala*, Koa Haole, in Hawaii. Hawaii Agricultural Experiment Station Research Bulletin No. 166. University of Hawaii, Honolulu, HI, USA. [ctahr.hawaii.edu/oc/freepubs/pdf/RB-166.pdf](https://ctahr.hawaii.edu/oc/freepubs/pdf/RB-166.pdf)
- Casanova-Lugo F; Petit-Aldana J; Solorio-Sánchez FJ; Parsons D; Ramirez-Avilés L. 2014. Forage yield and quality of *Leucaena leucocephala* and *Guazuma ulmifolia* in mixed and pure fodder banks systems in Yucatan, Mexico. Agroforestry Systems 88:29–39. doi: [10.1007/s10457-013-9652-7](https://doi.org/10.1007/s10457-013-9652-7)
- Chotchutima S; Tudsri S; Kangvansaichol K; Sripichitt P. 2016. Effects of sulfur and phosphorus application on the growth, biomass yield and fuel properties of leucaena [*Leucaena leucocephala* (Lam.) de Wit.] as bioenergy crop on sandy infertile soil. Agricultural and Natural Resources 50:54–59. doi: [10.1016/j.anres.2015.09.002](https://doi.org/10.1016/j.anres.2015.09.002)
- Costa NDL; Paulino VT; Magalhães JA. 2014. Effects of cutting regimes on forage yield and chemical composition of *Leucaena leucocephala*. PUBVET, Publicações em Medicina Veterinária e Zootecnia 8(20):1791. [bit.ly/3HYc7qI](https://bit.ly/3HYc7qI)
- Crounse RG; Maxwell JD; Blank H. 1962. Inhibition of growth of hair by mimosine. Nature 194:694–695. doi: [10.1038/194694b0](https://doi.org/10.1038/194694b0)
- Daehler CC; Denslow J. 2019. Weed risk assessment for Hawaii and Pacific Islands. [botany.hawaii.edu/faculty/daehler/wra/](https://botany.hawaii.edu/faculty/daehler/wra/)
- Dalzell SA; Burnett DJ; Dowsett JE; Forbes VE; Shelton HM. 2012. Prevalence of mimosine and DHP toxicity in cattle grazing *Leucaena leucocephala* pastures in Queensland, Australia. Animal Production Science 52:365–372. doi: [10.1071/AN11236](https://doi.org/10.1071/AN11236)
- Dalzell SA; Kerven GL. 1998. A rapid method for the measurement of *Leucaena* spp. proanthocyanidins by the proanthocyanidin (butanol/HCl) assay. Journal of the Science of Food and Agriculture 78:405–415. doi: [10.1002/\(SICI\)1097-0010\(199811\)78:3<405::AID-JSFA133>3.0.CO;2-G](https://doi.org/10.1002/(SICI)1097-0010(199811)78:3<405::AID-JSFA133>3.0.CO;2-G)
- Dewreede S; Wayman O. 1970. Effect of mimosine on the rat fetus. Teratology 3:21–27. doi: [10.1002/tera.1420030106](https://doi.org/10.1002/tera.1420030106)
- Ericson MC. 1985. Purification and properties of glutamine synthetase from spinach leaves. Plant Physiology 79:923–927. doi: [10.1104/pp.79.4.923](https://doi.org/10.1104/pp.79.4.923)
- Haliday MJ; Giles HE; Padmanabha J; McSweeney CS; Dalzell SA; Shelton HM. 2018. The efficacy of a cultured *Synergistes jonesii* inoculum to control hydroxypyridone toxicity in *Bos indicus* steers fed leucaena/grass diets. Animal Production Science 59:696–708. doi: [10.1071/AN17853](https://doi.org/10.1071/AN17853)
- Haliday MJ; Padmanabha J; McSweeney CS; Graham K; Shelton HM. 2013. Leucaena toxicity: a new perspective on the most widely used forage tree legume. Tropical Grasslands-Forrajes Tropicales 1:1–11. doi: [10.17138/tgft\(1\)1-11](https://doi.org/10.17138/tgft(1)1-11)
- Hamilton RI; Donaldson LE; Lambourne LJ. 1968. Enlarged thyroid glands in calves born to heifers fed a sole diet of *Leucaena leucocephala*. Australian Veterinary Journal 44:484. doi: [10.1111/j.1751-0813.1968.tb08984.x](https://doi.org/10.1111/j.1751-0813.1968.tb08984.x)
- Hayashi T; Yoshida K; Park YW; Konishi T; Baba K. 2004. Cellulose metabolism in plants. International Review of Cytology 247:1–34. doi: [10.1016/S0074-7696\(05\)47001-1](https://doi.org/10.1016/S0074-7696(05)47001-1)
- Hegarty MP; Lee CP; Christie GS; Court RD; Haydock KP. 1979. The goitrogen 3-Hydroxy-4. 1H- Pyridone, a ruminal metabolite from *Leucaena leucocephala*: effects in mice and rats. Australian Journal of Biological Sciences 32:27–40. doi: [10.1071/BI9790027](https://doi.org/10.1071/BI9790027)
- Honda MDH; Borthakur D. 2019. Mimosine concentration in *Leucaena leucocephala* under various environmental

- conditions. *Tropical Grasslands-Forrajes Tropicales* 7:164–172. doi: [10.17138/tgft\(7\)164-172](https://doi.org/10.17138/tgft(7)164-172)
- Honda MDH; Borthakur D. 2020. Mimosine facilitates metallic cation uptake by plants through formation of mimosine-cation complexes. *Plant Molecular Biology* 102:431–445. doi: [10.1007/s11103-019-00956-1](https://doi.org/10.1007/s11103-019-00956-1)
- Honda MDH; Borthakur D. 2021. Mimosine is a stress-response molecule that serves as both an antioxidant and osmolyte in Giant leucaena (*Leucaena leucocephala* subsp. *glabrata*) during environmental stress conditions. *Plant Stress* 2:100015. doi: [10.1016/j.stress.2021.100015](https://doi.org/10.1016/j.stress.2021.100015)
- Honda MDH; Ishihara KL; Pham DT; Borthakur D. 2018. Identification of drought-induced genes in Giant leucaena (*Leucaena leucocephala* subsp. *glabrata*). *Trees* 32:571–585. doi: [10.1007/s00468-018-1657-4](https://doi.org/10.1007/s00468-018-1657-4)
- Honda MDH; Ishihara KL; Pham DT; Borthakur D. 2019. Highly expressed genes in the foliage of Giant leucaena, *Leucaena leucocephala* subsp. *glabrata*, a nutritious fodder legume in the tropics. *Plant Biosystems* 154:107–116. doi: [10.1080/11263504.2019.1578283](https://doi.org/10.1080/11263504.2019.1578283)
- Ishihara KL; Honda MDH; Bageel A; Borthakur D. 2018. *Leucaena leucocephala*: a leguminous tree suitable for eroded habitats of Hawaiian Islands. In: Dagar J; Singh A, eds., *Ravine Lands: Greening for Livelihood and Environmental Security*. Springer, Singapore. p. 413–431. doi: [10.1007/978-981-10-8043-2\\_18](https://doi.org/10.1007/978-981-10-8043-2_18)
- Jones RJ. 1981. Does ruminal metabolism of mimosine explain the absence of *Leucaena* toxicity in Hawaii? *Australian Veterinary Journal* 57:55–56. doi: [10.1111/j.1751-0813.1981.tb07097.x](https://doi.org/10.1111/j.1751-0813.1981.tb07097.x)
- Joshi HS. 1968. The effect of feeding *Leucaena leucocephala* (Lam.) de Wit on reproduction in rats. *Australian Journal of Agricultural Research* 19:341–352. doi: [10.1071/AR9680341](https://doi.org/10.1071/AR9680341)
- Kienzle E; Schrag I; Butterwick R; Opitz B. 2001. Calculation of gross energy in pet foods: new data on heat combustion and fibre analysis in a selection of foods for dogs and cats. *Journal of Animal Physiology and Animal Nutrition* 85:148–157. doi: [10.1046/j.1439-0396.2001.00311.x](https://doi.org/10.1046/j.1439-0396.2001.00311.x)
- López F; García MM; Yáñez R; Tapias R; Fernández M; Díaz MJ. 2008. *Leucaena* species valoration for biomass and paper production in 1 and 2 year harvest. *Bioresource Technology* 99:4846–4853. doi: [10.1016/j.biortech.2007.09.048](https://doi.org/10.1016/j.biortech.2007.09.048)
- Lowry JB; Maryanto; Tangendjaja B. 1983. Autolysis of mimosine to 3-hydroxy-4-(1)pyridone in green tissues of *Leucaena leucocephala*. *Journal of the Science of Food and Agriculture* 34:529–533. doi: [10.1002/jsfa.2740340602](https://doi.org/10.1002/jsfa.2740340602)
- Meng DH; Du RR; Chen LZ; Li MT; Liu F; Hou J; Shi YK; Wang FS; Sheng JZ. 2019. Cascade synthesis of uridine-5'-diphosphate glucuronic acid by coupling multiple whole cells expressing hyperthermophilic enzymes. *Microbial Cell Factories* 18:118. doi: [10.1186/s12934-019-1168-z](https://doi.org/10.1186/s12934-019-1168-z)
- Mertens DR. 1987. Predicting intake and digestibility using mathematical models of ruminal function. *Journal of Animal Science* 64:1548–1558. doi: [10.2527/jas1987.6451548x](https://doi.org/10.2527/jas1987.6451548x)
- Mishra K; Ojha H; Chaudhury NK. 2012. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chemistry* 130:1036–1043. doi: [10.1016/j.foodchem.2011.07.127](https://doi.org/10.1016/j.foodchem.2011.07.127)
- Mullen BF; Gutteridge RC. 2002. Wood and biomass production of *Leucaena* in subtropical Australia. *Agroforestry Systems* 55:195–205. doi: [10.1023/A:1020570115918](https://doi.org/10.1023/A:1020570115918)
- Negi VS; Bingham JP; Li QX; Borthakur D. 2013. *midD*-encoded 'rhizomimosinase' from *Rhizobium* sp. strain TAL145 is a C–N lyase that catabolizes L-mimosine into 3-hydroxy-4-pyridone, pyruvate and ammonia. *Amino Acids* 44:1537–1547. doi: [10.1007/s00726-013-1479-z](https://doi.org/10.1007/s00726-013-1479-z)
- Negi VS; Bingham JP; Li QX; Borthakur D. 2014. A carbon-nitrogen lyase from *Leucaena leucocephala* catalyzes the first step of mimosine degradation. *Plant Physiology* 164:922–934. doi: [10.1104/pp.113.230870](https://doi.org/10.1104/pp.113.230870)
- Negi VS; Borthakur D. 2016. Heterologous expression and characterization of mimosinase from *Leucaena leucocephala*. In: Fett-Neto A, eds. *Biotechnology of Plant Secondary Metabolism. Methods in Molecular Biology*, vol 1405. Humana Press, New York, NY, USA . p. 59–77. doi: [10.1007/978-1-4939-3393-8\\_7](https://doi.org/10.1007/978-1-4939-3393-8_7)
- Obregón-Cano S; Moreno-Rojas R; Jurado-Millán AM; Cartea-González ME; De Haro-Bailón A. 2019. Analysis of the acid detergent fibre content in turnip greens and turnip tops (*Brassica rapa* l. subsp. *rapa*) by means of near-infrared reflectance. *Foods* 8(9):364. doi: [10.3390/foods8090364](https://doi.org/10.3390/foods8090364)
- Panjaitan T; Fordyce G; Poppi DP. 2010. Breeding *Bos javanicus* d'Alton cattle in eastern Indonesia: cattle control, diets, draught use and feeding. In: Santosa KA, ed. *Proceedings of the 5th International Seminar on Tropical Animal Production*, Yogyakarta, Indonesia, October 19–22, 2010. p. 478–482. [bit.ly/34BrBT5](https://doi.org/10.1007/978-1-4939-3393-8_7)
- Pathak PS; Patil BD. 1983. *Leucaena* Research at the Indian Grassland and Fodder Research Institute (IGFRI). In: *Leucaena research in the Asian – Pacific Region. Proceedings of a Workshop held in Singapore*, 23–26 November 1982. Nitrogen Fixing Tree Association & International Development Research Centre, Canada.
- Reed JD. 2001. Effects of proanthocyanidins on digestion of fiber in forages. *Journal of Range Management* 54:466–473. doi: [10.2307/4003118](https://doi.org/10.2307/4003118)
- Rengsirikul K; Kanjanakuha A; Ishii Y; Kangvansaichol K; Sripichitt P; Punsuvon V; Vaithanomsat P; Nakamane G; Tudsri S. 2011. Potential forage and biomass production of newly introduced varieties of leucaena [*Leucaena leucocephala* (Lam.) de Wit.] in Thailand. *Grassland Science* 57:94–100. doi: [10.1111/j.1744-697X.2011.00213.x](https://doi.org/10.1111/j.1744-697X.2011.00213.x)
- Robbins NS; Pharr DM. 1988. Effect of restricted root growth on carbohydrate metabolism and whole plant growth of *Cucumis sativus* L. *Plant Physiology* 87:409–413. doi: [10.1104/pp.87.2.409](https://doi.org/10.1104/pp.87.2.409)

- Rodrigues-Corrêa KCS; Honda MDH; Borthakur D; Fett-Neto AG. 2019. Mimosine accumulation in *Leucaena leucocephala* in response to stress signaling molecules and acute UV exposure. *Plant Physiology and Biochemistry* 135:432–440. doi: [10.1016/j.plaphy.2018.11.018](https://doi.org/10.1016/j.plaphy.2018.11.018)
- Sanginga N; Mulongoy K; Ayanaba A. 1989. Nitrogen fixation of field-inoculated *Leucaena leucocephala* (Lam.) de Wit estimated by the  $^{15}\text{N}$  and the difference methods. *Plant and Soil* 117:269–274. doi: [10.1007/BF02220721](https://doi.org/10.1007/BF02220721)
- Shay PE; Trofymow JA; Constabel CP. 2017. An improved butanol-HCl assay for quantification of water-soluble, acetone:methanol-soluble, and insoluble proanthocyanidins (condensed tannins). *Plant Methods* 13:63. doi: [10.1186/s13007-017-0213-3](https://doi.org/10.1186/s13007-017-0213-3)
- Shelton HM; Brewbaker JL. 1994. *Leucaena leucocephala* - the most widely used forage tree legume. In: *Forage tree legumes in tropical agriculture*. CAB International, London, UK. p. 15–29. [cabi.org/isc/abstract/19940601654](https://cabi.org/isc/abstract/19940601654)
- Shelton HM; Kerven GL; Dalzell SA. 2019. An update on leucaena toxicity: Is inoculation with *Synergistes jonesii* necessary? *Tropical Grasslands-Forrajes Tropicales* 7:146–153. doi: [10.17138/tgft\(7\)146-153](https://doi.org/10.17138/tgft(7)146-153)
- Soedarjo M; Borthakur D. 1996. Simple procedures to remove mimosine from young leaves, pods and seeds of *Leucaena leucocephala* used as food. *International Journal of Food Science and Technology* 31:97–103. doi: [10.1111/j.1365-2621.1996.24-321.x](https://doi.org/10.1111/j.1365-2621.1996.24-321.x)
- Soedarjo M; Hemscheidt TK; Borthakur D. 1994. Mimosine, a toxin present in the tree legume *Leucaena*, induces a mimosine-degrading enzyme activity in some *Rhizobium* strains. *Applied Environmental Microbiology* 60:4268–4272. doi: [10.1128/aem.60.12.4268-4272.1994](https://doi.org/10.1128/aem.60.12.4268-4272.1994)
- ter Meulen U; Struck S; Schulke E; El-Marith EA. 1979. A review on the nutritive value and toxic aspects of *Leucaena leucocephala*. *Tropical Animal Production* 4:113–126. [fao.org/ag/aga/agap/frg/tap42/4\\_2\\_1.pdf](https://fao.org/ag/aga/agap/frg/tap42/4_2_1.pdf)
- Tsugama D; Liu S; Takano T. 2011. A rapid chemical method for lysing *Arabidopsis* cells for protein analysis. *Plant Methods* 7:22. doi: [10.1186/1746-4811-7-22](https://doi.org/10.1186/1746-4811-7-22)
- Tudsri S; Chotchutima S; Nakamane K; Kangwansaichol K. 2019. Dual use of leucaena for bioenergy and animal feed in Thailand. *Tropical Grasslands-Forrajes Tropicales* 7:193–199. doi: [10.17138/TGFT\(7\)193-199](https://doi.org/10.17138/TGFT(7)193-199)
- Van den Beldt RJ. 1983. *Leucaena leucocephala* (Lam.) de Wit for wood production. Ph.D. Thesis. University of Hawaii, Honolulu, HI, USA. [hdl.handle.net/10125/56461](https://hdl.handle.net/10125/56461)
- Van den Beldt RJ; Brewbaker JL. 1980. *Leucaena* wood production trials in Hawaii. *Leucaena Newsletter* 1:55.
- Yemm EW; Willis AJ. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal* 57:508–514. doi: [10.1042/bj0570508](https://doi.org/10.1042/bj0570508)
- Youkhana AH; Idol TW. 2009. Tree pruning mulch increases soil C and N in a shaded coffee agroecosystem in Hawaii. *Soil Biology and Biochemistry* 41:2527–2534. doi: [10.1016/j.soilbio.2009.09.011](https://doi.org/10.1016/j.soilbio.2009.09.011)
- Youkhana AH; Idol TW. 2011. Allometric models for predicting above- and belowground biomass of *Leucaena*-KX2 in shaded coffee agroecosystems in Hawaii. *Agroforestry Systems* 83:331–345. doi: [10.1007/s10457-011-9403-6](https://doi.org/10.1007/s10457-011-9403-6)
- Youkhana AH; Idol TW. 2016. *Leucaena*-KX2 mulch additions increase growth, yield and soil C and N in a managed full-sun coffee system in Hawaii. *Agroforestry Systems* 90:325–337. doi: [10.1007/s10457-015-9857-z](https://doi.org/10.1007/s10457-015-9857-z)
- Youkhana A; Idol T. 2018. Cut-and-carry for sustaining productivity and carbon sequestration in agroforestry systems: Coffee-*Leucaena* example. In: Dagar J; Tewari V, eds. *Agroforestry*. Springer, Singapore. doi: [10.1007/978-981-10-7650-3\\_22](https://doi.org/10.1007/978-981-10-7650-3_22)
- Zarin MA; Wan HY; Isha A; Armania N. 2016. Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from *Leucaena leucocephala* hybrid-Rendang. *Food Science and Human Wellness*. 5: 65–75. doi: [10.1016/j.fshw.2016.02.001](https://doi.org/10.1016/j.fshw.2016.02.001)
- Zhong H; Xue Y; Lu X; Shao Q; Cao Y; Wu Z; Chen G. 2018. The effects of different degrees of procyanidin polymerization on the nutrient absorption and digestive enzyme activity in mice. *Molecules* 23:2916. doi: [10.3390/molecules23112916](https://doi.org/10.3390/molecules23112916)

(Received for publication 19 October 2020; accepted 20 November 2021; published 31 January 2022)

© 2022



*Tropical Grasslands-Forrajes Tropicales* is an open-access journal published by *International Center for Tropical Agriculture (CIAT)*, in association with *Chinese Academy of Tropical Agricultural Sciences (CATAS)*. This work is licensed under the Creative Commons Attribution 4.0 International (CC BY 4.0) license.