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. HONDA¹, ADEL YOUKHANA², TRAVIS IDOL² AND DULAL BORTHAKUR¹

¹Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI, USA. manoa.hawaii.edu
²Department of Natural Resources and Environmental Management, University of Hawaii at Manoa, Honolulu, HI, USA. 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 aspect 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...
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.
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\(^{3+}\), Cu\(^{2+}\), Zn\(^{2+}\) 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 in animals that include reduced feed intake, goiter, 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

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

<table>
<thead>
<tr>
<th>Variety</th>
<th>Cross/parentage</th>
<th>Edible biomass (DM)</th>
<th>Inedible biomass (DM)</th>
<th>Total biomass (DM)</th>
<th>Edible biomass (FM)</th>
<th>Inedible biomass (FM)</th>
<th>Total biomass (FM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giant</td>
<td>L. leucocephala subsp. leucocephala</td>
<td>32.9, 33.3, 33.9</td>
<td>34.0, 34.9</td>
<td>37.1, 37.6, 38.6</td>
<td>39.8, 40.3</td>
<td>93.9, 99.5</td>
<td>149.3, 152.7</td>
<td>202.0</td>
</tr>
<tr>
<td>leucaena1</td>
<td>L. leucocephala subsp. glabrata (Rose) Zárate</td>
<td>5.9, 6.0, 6.1</td>
<td>15.1, 17.4, 17.5</td>
<td>22.8, 23.1</td>
<td>24.1, 24.5</td>
<td>18.9,19.8</td>
<td>21.8, 49.7</td>
<td>55.6</td>
</tr>
<tr>
<td>6.6, 6.8, 6.9</td>
<td>L. pallida x L. leucocephala</td>
<td>17.9, 21.2, 23.1</td>
<td>21.7, 22.9</td>
<td>24.8, 28.1</td>
<td>29.0, 33.9</td>
<td>32.3, 33.0</td>
<td>36.8, 42.5</td>
<td>202.0</td>
</tr>
</tbody>
</table>

1Includes K8, K636, Tarramba, Peru, Cunningham, Salvador and other types.

### Table 2. Leucaena varieties analyzed.

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

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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 H₂O 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 Ciocalteau 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.

### 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>
<thead>
<tr>
<th>Variety</th>
<th>Dry matter (%)</th>
<th>Crude protein (%DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common leucaena</td>
<td>30.2 ± 0.5</td>
<td>10.9 ± 0.3</td>
</tr>
<tr>
<td>Giant leucaena K636</td>
<td>29.9 ± 1.2</td>
<td>17.6 ± 4.4</td>
</tr>
<tr>
<td>Giant leucaena KX2</td>
<td>26.8 ± 0.8</td>
<td>14.4 ± 1.0</td>
</tr>
<tr>
<td>Giant leucaena KX3</td>
<td>29.4 ± 0.6</td>
<td>16.8 ± 0.3</td>
</tr>
<tr>
<td>Giant leucaena KX4</td>
<td>26.7 ± 1.2</td>
<td>11.1 ± 0.5</td>
</tr>
<tr>
<td>Giant leucaena KX5</td>
<td>27.8 ± 0.8</td>
<td>15.6 ± 1.0</td>
</tr>
<tr>
<td>Giant leucaena KX7</td>
<td>32.1 ± 0.3</td>
<td>11.2 ± 0.9</td>
</tr>
<tr>
<td>All</td>
<td>28.9 ± 0.4</td>
<td>13.9 ± 2.9</td>
</tr>
</tbody>
</table>

### 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 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.

_Tropical Grasslands-Forrajes Tropicales (ISSN: 2346-3775)_
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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 leaves (18.5%) (Figure 6a). Macerated leaves also had a lower crude fat concentration (3.8% DM) than the

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**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.
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).
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 (± s.e.), fat 9 kcal/g and ADF and NDF 5 kcal/g.

<table>
<thead>
<tr>
<th></th>
<th>Calculated gross energy concentration (total kcal/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unprocessed Control</td>
</tr>
<tr>
<td>Protein</td>
<td>743 ± 14</td>
</tr>
<tr>
<td>Fat</td>
<td>494 ± 05</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>376 ± 01</td>
</tr>
<tr>
<td>ADF</td>
<td>665 ± 08</td>
</tr>
<tr>
<td>NDF</td>
<td>1,455 ± 46</td>
</tr>
<tr>
<td>Total</td>
<td>3,733 ± 55</td>
</tr>
</tbody>
</table>

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 (Dachler 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 β-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 β-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](image-url)  
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 (C₆H₁₂O₆; 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.
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(Note of the editors: All hyperlinks were verified 9 December 2021).


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Tropical Grasslands-Forrages Tropicales (ISSN: 2346-3775)
Tropical Grasslands-Forrajes Tropicales (ISSN: 2346-3775)

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

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