

# Comparison Of Mimosine Content And Nutritive Values Of Neolamarckia Cadamba And Leucaena Leucocephala With Medicago Sativa As Forage Quality Index

Mohamed Zaky Zayed, Mohamed Abdallah Zaki, Fasihuddin Badruddin Ahmad, Wei-Seng Ho, Shek-Ling Pang

**Abstract:** A study was conducted to determine the mimosine content and the nutritive values of *Neolamarckia cadamba* and *Leucaena leucocephala* in comparison to *Medicago sativa* (alfalfa hay) as forage quality index. A total of 22 *N. cadamba* and 35 *L. leucocephala* seedlings were analyzed to determine the mimosine content after 6 months of planting. It was noted that the mimosine content was highest in *L. leucocephala* (1.6%) and lowest in *N. cadamba* (0.03%) in comparison to *M. sativa* which has no mimosine content. Crude protein content was 23.48%, 20.90% and 14.83% for *L. leucocephala*, *N. cadamba* and *M. sativa*, respectively. The crude fiber was maximum in *M. sativa* (27.23%) and minimum in *L. leucocephala* (18.77%). Crude protein, crude fat, gross energy, protein to energy (P/E) ratio, organic matter and total ash in *N. cadamba* was higher compared to *M. sativa*. *L. leucocephala* was lower in nitrogen free extract, crude fiber and total ash compared to *N. cadamba*. Results from this study clearly indicate that *N. cadamba* has high forage quality and comparable to the traditional *L. leucocephala* and *M. sativa* as forage for ruminant and non-ruminants.

**Index Terms:** Neolamarckia cadamba, Leucaena leucocephala, Medicago sativa, mimosine, nutritive value, forage quality index

## 1 INTRODUCTION

The need to develop cheap and readily available alternative feeding materials to support livestock growth has become imperative. Leaf protein sources obtained in leaf vegetables, legume trees, fodder trees and shrubs as feed resources to all classes of livestock offer tremendous potentials and received increasing attention [1], [2], [3]. Mimosine is a free amino-acid very often present in certain legume plants which include *Leucaena leucocephala*. Mimosine and its degradation product 3-hydroxy-4(1H)-pyridone (DHP) are both toxic when ingested by herbivores and therefore, its presence limits the use of the leaves and seeds in feed for mono-gastric animals since it affects thyroid function, leading to poor growth and ultimately death in both ruminants and non-ruminants [4].

*Leucaena leucocephala* or locally known as petai belalang belongs to family Leguminosae. It is valued as an excellent protein source for cattle fodder, consumed browsed or harvested, mature or immature, green or dry. The nutritive value is equal to or superior to *Medicago sativa* (alfalfa) [5] and therefore, it is often being described as the alfalfa of the tropics. All parts of *L. leucocephala* are edible to animals, including leaves, young stem, flowers, young and mature pods, and seeds [5]. *Leucaena* foliage (leaflets plus stems) contains both nutrients and roughage and makes a ruminant feed roughly comparable to alfalfa forage. It is a rich source of protein (15-38%), produce up to 20 metric tons of dry matter per ha and the foliage is highly digestible (60-70%) [6]. *Neolamarckia cadamba* or locally known as kelampayan belongs to family Rubiaceae. It is one of the most frequently planted trees in the tropics and suitable for ornamental use and agroforestry practices [7]. In fact, it has been selected as one of the plantation tree species in forest rehabilitation projects in Malaysia due to its short rotation period [8], [9], [10], [11]. Under normal conditions, it reaches a height of 17 m and a diameter of 25 cm at breast height (dbh) within 9 years. It is one of the best sources of raw material for the plywood industry, besides pulp and paper production. It can be used as a shade tree for dipterocarp line planting, whilst its leaves and barks have medical applications. The dried barks can be used to relieve fever and as a tonic, whereas a leaf extract can serve as a mouth wash [12]. Other than medical applications, its leaves have also been used as fodder to cattle [7], but so far no scientific study has been done which may support its use in traditional forage. Thus, the present study was carried out to determine the nutritive value of *N. cadamba* and *L. leucocephala* with *M. sativa* as forage quality index. The mimosine content was also determined by using high-pressure liquid chromatography (HPLC) for both species.

- Dr. Wei-Seng Ho - \*Corresponding Author Forest Genomics and Informatics Laboratory (fGiL), Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak.  
E-mail: [wsho@frst.unimas.my](mailto:wsho@frst.unimas.my) / [howeiseng@gmail.com](mailto:howeiseng@gmail.com)
- Dr. Fasihuddin Badruddin Ahmad - Department of Chemistry, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak.
- Dr. Shek-Ling Pang - Applied Forest Science and Industry Development (AFSID), Sarawak Forestry Corporation, 93250 Kuching, Sarawak.
- Dr. Mohamed Abdallah Zaki - Department of Animal Production, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.
- Mohamed Zaky Zayed - Forest Genomics and Informatics Laboratory (fGiL), Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak / Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

## 2 MATERIALS AND METHODS

### 2.1 Mimosine content

The fresh leaves of *N. cadamba* and *L. leucocephala* were harvested at the age of seven months after planting. 1 g of the fresh leaves was added with 10 ml of 0.1 N HCl in a flask. The mixture was vortexed for 1-2 minutes and then extracted for 24 hours. HPLC (Shimadzu CTO-2A) UV-Vis detector analysis was performed on analytical column C18 (4.6 X 150 mm, 5 mM) at 60°C. A total of 20µl of sample solution was injected into the column and eluted with a mobile phase of 0.2% (w/v) orthophosphoric acid, and detected using UV at 280 nm based on the procedure of Puchala et al. [13].

### 2.2 Nutritive value

Leaves samples were ground into fine powder by using a grinder. The samples were analyzed for dry weight (DW), crude protein (CP), crude fat (EE), crude fiber (CF) and total ash (TA) as described by the Association of Official Analytical Chemists (AOAC) [14]. Moisture was determined by drying the samples at 105°C overnight and the loss in weight was reported as a percentage of moisture. Nitrogen free extract in the samples were calculated using the following equation: NFE = 100 - (Moisture + CP + EE + CF). Gross energy was calculated as 5.65, 9.45 and 4.12 Kcal per 100 gram of protein, lipid and carbohydrate, respectively.

## 3 RESULTS AND DISCUSSION

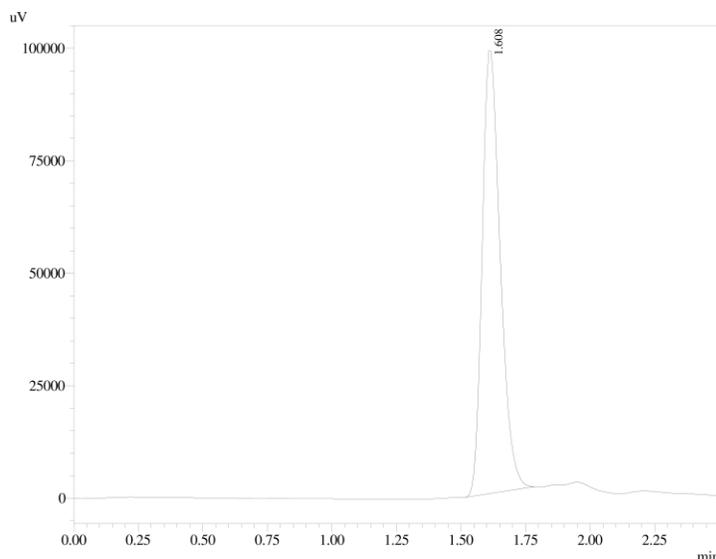
A total of 22 *N. cadamba* and 35 *L. leucocephala* seedlings were analyzed in the present study. Mimosine content was analyzed by using HPLC and the results are given in Table 1. The mimosine content was highest in *L. leucocephala* (1.60%) (Fig. 1) and lowest in *N. cadamba* (0.03%) (Fig. 2). The mimosine content in *N. cadamba* was much lower than *L. leucocephala*, while *M. sativa* (alfalfa) did not contain any mimosine [15]. Values obtained for mimosine content of fresh *L. leucocephala* leaves at the age of 7 months were nearly similar to those reported by EL-Ashry et al. [16], with mimosine content of 1.92% for fresh *L. leucocephala* leaves at the age of 7 months. Similar results were also reported by Mutayoba et al. [17], with mimosine content of 1.89% for *L. leucocephala* leaves. The results are comparable with the results obtained by Deshmukh et al. [18] and Silva and Haag [19], who stated that the mimosine content depends on the stage of plant growth being maximum on the thirtieth day (7.1%) and progressively decreased by 45 days (6.0%) and 60 days (4.2%) of growth. Cutting the plant had an effect as well, with mimosine content ranging from 2.5% to 4.9% and from 1.4% to 3.4%, in the second and third cuttings, respectively [20].

**TABLE 1**

MIMOSINE CONTENT (%) IN *N. CADAMBA* AND *L. LEUCOCEPHALA* LEAVES

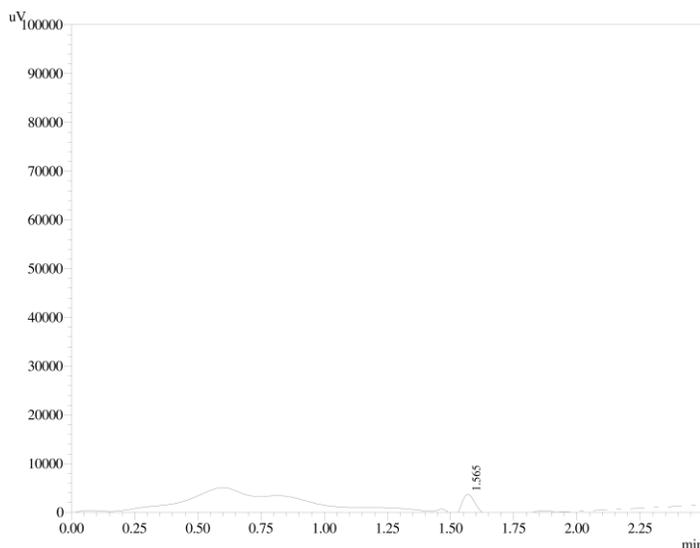
Species	Mimosine Content (%)
<i>L. leucocephala</i>	1.60 <sup>a</sup>
<i>N. cadamba</i>	0.03 <sup>b</sup>

LSD<sub>0.05</sub> means in the same column within each item having different superscript are significantly different ( $p < 0.05$ )



Name	Peak#	Ret. Time	Area	Height	Area %	Height %	Conc.	Units
RT1.629	1	1.608	480671	98517	100.000	100.000	16.200	mg/L
	Total		480671	98517	100.000	100.000		

**Fig. 1.** Chromatogram of mimosine content in *L. leucocephala* leaves



Name	Peak#	Ret. Time	Area	Height	Area %	Height %	Conc.	Units
RT1.634	1	1.565	17188	4470	100.000	100.000	0.572	mg/L
	Total		17188	4470	100.000	100.000		

**Fig. 2.** Chromatogram of mimosine content in *N. cadamba* leaves

Table 2 shows the nutritive value of *N. cadamba* and *L. leucocephala* leaves. *L. leucocephala* showed the highest in crude protein and crude fat, meanwhile *N. cadamba* was highest in crude fiber and total ash than *L. leucocephala*. This result is comparable to *M. sativa* with the nutritive value of 14.83%, 27.23%, 2.15%, 6.27%, 90.24%, 39.76%, 267.92 kcal/100g and 55.35 mg cp/kcal for crude protein (CP), crude fiber (CF), crude fat (EE), total ash (TA), organic matter (OM), nitrogen-free extract (NFE), gross energy and crude

protein/gross energy ratio (P/E ratio), respectively as reported by Babth et al. [21]. Crude protein content was 23.48%, 20.90%, 14.83% and 20.81% for *L. leucocephala*, *N. cadamba*, *M. sativa* (alfalfa) [21] and green alfalfa [22], respectively. The crude fibre showed the highest in *M. sativa* (27.23%), intermediate in *N. cadamba* (20.27%) and the lowest in *L. leucocephala* (18.77%). Crude protein, crude fat, gross energy, protein to energy ratio, organic matter and total ash in *N. cadamba* was higher compared to *M. sativa*. *L. leucocephala* was lower in nitrogen free extract, crude fiber and total ash compared to *N. cadamba*. The results from this study indicate that *N. cadamba* has high forage quality in comparison to the traditional *L. leucocephala* and *M. sativa* as forage for ruminants and non-ruminants. The crude protein (CP) value was high in both species which is in agreement with the observation of Waldroup and Smith [23] that multipurpose trees contain 20.0% CP or above in their leaves. The CP contents were, however, higher than the values of leaf meals obtained from grasses and vegetable shrubs which seldom exceed 15.0g/100g [24], [25]. The total ash contents were high which indicates that inorganic elements are

substantial in the plants [26]. The nutritive values obtained from *L. leucocephala* leaves are comparable to those published results. Atawodi [27] analyzed leaves of *L. leucocephala* and found that the nutritive values were 22.76%, 22.29%, 4.60% and 9.73% for crude protein, crude fiber, crude fat and total ash, respectively. Sallam [28] reported that *L. leucocephala* contained 8.59% ash, 91.41% organic matter, 19.93% crude protein, 11.06% crude fiber, 0.97% ether extract and 59.45% nitrogen free extract. Aganga and Tshwenyane [29] also found that the average crude fiber of the whole plant of *L. leucocephala* was 11.84%, with the average neutral detergent fiber, acid detergent fiber (ADF) and acid detergent lignin (ADL) were 34.5%, 24.7% and 15.5%, respectively (on dry matter basis). Wheeler et al. [30] found that the dry matter digestibility (DMD) of *L. leucocephala* was 57.7%, while crude protein of *L. leucocephala* based on dry matter was 29.5%. Similar results were also observed by Maw [31] on the nutritive value of some foliage trees locally available in Myanmar. He reported that the nutritive values were in the

TABLE 2

THE NUTRITIVE VALUE OF *N. CADAMBA* AND *L. LEUCOCEPHALA* LEAVES IN COMPARISON TO *M. SATIVA*

Species	CP (%)	CF (%)	EE (%)	TA (%)	OM (%)	NFE (%)	GE (kcal/100g)	P/E (mg cp/kcal)
<i>N. cadamba</i>	20.90 <sup>b</sup>	20.27 <sup>a</sup>	2.60 <sup>b</sup>	9.76 <sup>a</sup>	90.32 <sup>a</sup>	36.78 <sup>a</sup>	294.28 <sup>b</sup>	71.02 <sup>a</sup>
<i>L. leucocephala</i>	23.48 <sup>a</sup>	18.77 <sup>b</sup>	3.31 <sup>a</sup>	9.09 <sup>b</sup>	90.89 <sup>a</sup>	36.24 <sup>a</sup>	313.25 <sup>a</sup>	74.96 <sup>a</sup>
<i>M. sativa</i> [21]	14.83	27.23	2.15	6.27	90.24	39.76	267.92	55.35

LSD<sub>0.05</sub> means in the same column within each item having different superscript are significantly different ( $p < 0.05$ )

range of dry matter (8.30% - 86.50%), organic matter (66.89% - 98.05%), crude protein (8.03% - 32.43%), neutral detergent fibre (16.93% - 71.10%) and acid detergent fiber (7.37% - 53.10%).

#### 4 CONCLUSION

This study has confirmed that *N. cadamba* leaves have high forage quality due to its high protein and low mimosine contents, besides its medicinal values. Its nutritive value is comparable or superior to the traditional *L. leucocephala* and *M. sativa* as forage for ruminants and non-ruminants. In fact, this is the first report on the nutritive value of *N. cadamba* leaves. It is hoped that this result could pave the way for developing a cost effective livestock feed with high protein content so that we may achieve economic benefits of great significance in future.

#### ACKNOWLEDGEMENTS

This work is part of the joint Industry-University Partnership Programme, a research programme funded by the Sarawak Forestry Corporation (SFC), Sarawak Timber Association (STA) and Universiti Malaysia Sarawak under grant no. 02(DPI09)832/2012(1), RACE/a(2)/884/2012(02) and GL(F07)/06/2013/STA-UNIMAS(06).

#### REFERENCES

- [1] P.A. Aye, "Production of multinutrient blocks for ruminant and alcohol from the waste products of *Leucaena leucocephala* and *Gliricidia sepium* leaves using local technologies," PhD thesis, Dept. of Animal Production and Health, Federal University of Technology, Akure, Nigeria, 2007.
- [2] A.O. Fasuyi and O. A. Kehinde, "Effect of cellulose glucanase xylanase combination on the nutritive value of *Telfairia occidentalis* leaf meal in broiler diets," *Journal of Cell and Animal Biology*, vol. 3, no. 11, pp.188-195, 2009.
- [3] V.O. Asaolu, R.T. Binuomote, J.O. Akinlade, O.J. Oyelami and K.O. Kolapo, "Utilization of *Moringa oleifera* fodder combinations with *Leucaena leucocephala* and *Gliricidia sepium* fodders for west African dwarf goats," *International Journal of Agriculture Research*, vol. 6, pp. 607-619, 2011.
- [4] N. Chanchay and N. Poosaran, "The reduction of mimosine and tannin contents in leaves of *Leucaena leucocephala*," *Asian Journal of Food and Agro-Industry*, Special Issue, S137-S144, 2009.
- [5] R.C. Mendoza, "Herbage crude protein and digestible dry matter yield of Ipil-Ipil (*Leucaena latifolia* Cv.

- Peru,” Hedge Rows-Animal scientific Convention of the Philippines Society of Animal Science, 1975.
- [6] J.L. Brewbaker, “*Leucaena leucocephala* - a versatile nitrogen fixing tree,” Fact Sheet 97-06. Winrock International, Morrilton, AK, 1997.
- [7] I. Soerianegara and R.H.M.J. Lemmens, “Plant resources of South-east Asia 5 (1): Timber trees: Major commercial timbers,” Pudoc Scientific Publishers, Wageningen, Netherlands, 1993.
- [8] B.L. Tchin, W.S. Ho, S.L. Pang and J. Ismail, “Association genetics of the cinnamyl alcohol dehydrogenase (CAD) and cinnamate 4-hydroxylase (C4H) genes with basic wood density in *Neolamarckia cadamba*,” *Biotechnology*, vol. 11, no. 6, pp. 307-317, 2012.
- [9] P.S. Lai, W.S. Ho and S.L. Pang, “Development, characterization and cross-species transferability of expressed sequence tag-simple sequence repeat (EST-SSR) markers derived from kelampayan tree transcriptome,” *Biotechnology*, vol. 12, no. 6, pp.225-235, 2013.
- [10] S.Y. Tiong, W.S. Ho, S.L. Pang and J. Ismail, “Nucleotide diversity and association genetics of xyloglucan endotransglycosylase/ hydrolase (XTH) and cellulose synthase (CesA) genes in *Neolamarckia cadamba*,” *Journal of Biological Sciences*, vol. 14, no. 4, pp. 267-375, 2014.
- [11] W.S. Ho, S.L. Pang and A. Julaihi, “Identification and analysis of expressed sequence tags present in xylem tissues of kelampayan (*Neolamarckia cadamba* (Roxb.) Bosser),” *Physiology and Molecular Biology of Plants*, published online, DOI 10.1007/s12298-014-0230-x, 2014.
- [12] World Agroforestry Centre, “A tree species reference and selection guide: *Anthocephalus cadamba*,” (<http://www.worldagroforestrycentre.org/sea/Products/AFDbases/af/asp/SpeciesInfo.asp?SpID=17933>), 2004.
- [13] R. Puchala, J.J. Davis and T. Sahlu, “Determination of mimosine and 3,4- dihydroxypyridine in milk and plasma of goats,” *Journal of Chromatography B: Biomedical Applications*, vol. 685, pp. 375-378, 1996.
- [14] A.O.A.C, “Official methods of analysis,” 15<sup>th</sup> Edition, Association of official Analytical chemists, Washington D.C., 2000.
- [15] NAS (National Academy of Sciences), “*Leucaena*: Promising forage and tree crop for the tropics,” Washington D.C., USA, 1977.
- [16] M.A. El- Ashry, H.M. Khattab, F.R. Helal, M.M. Shoukry and S.A. Abo El-Nour, “*Leucaena leucocephala*: New forage for farm animals in Egypt,” *Egypt. Jour. Anim. Prod.*, vol. 30, no. 1, pp. 83-91, 1993.
- [17] S.K. Mutayoba, B.M. Mutayoba and P. Okot, “The performance of growing pullets fed diets with varying energy and *Leucaena* leaf meal levels,” *Livestock Research for Rural Development*, vol. 15, no. 8 (<http://www.lrrd.org/lrrd15/8/muta158.htm>), 2003.
- [18] A.P. Deshmukh, D.S. Doiphode, J.S. Desale and V.D. Deshmukh, “Chemical composition of subabul as influenced by types and growth stages,” *J Maharashtra Agric Univ*, vol. 12, pp. 25-31, 1987.
- [19] D.H. Silva and H.P. Haag, “Mineral nutrition of tropical legumes III. Concentration and accumulation of macronutrients and determination of dry matter digestibility *in vivo* of *Leucaena leucocephala* (Lam.) de Wit cv. Peru as a function of age,” *Escola Superior de Agricultral "Lutz de Queiroz" Boletim, Univ. Sao Paulo*, vol. 39, pp. 505-38, 1982.
- [20] R. Kasthuri and S. Sadasivam, “Note on the effect of cutting on the level of mimosine in *Leucaena*,” *Indian Forest*, vol. 117, pp. 577-579, 1991.
- [21] M. Babth, W.V. Noot and J.L. Cason, “A comparison of the nutritive value of alfalfa hay with brome grass and reed canary grass hays at various levels of nitrogen fertilization,” *Journal of Nutrition*, vol. 68, pp: 383-391, 1958.
- [22] D. Andueza, F. Muñoz and A. Garrido, “The prediction of the nutritive value of Mediterranean alfalfa forage by NIRS,” *Options Méditerranéennes: Série A. Séminaires Méditerranéens*, vol. 45, pp: 199 -203, 2001.
- [23] P.W. Waldroup and K. Smith, “Fact sheet soybean use Poultry,” Soybean Meal Information Center, 2008.
- [24] M.A. Jabbar, L. Reynolds, A. Larbi and J. Smith, “Nutritional and economic benefits of *Leucaena* and *Gliricidia* as feed supplements for small ruminants in humid West Africa,” *Tropical Animal Health and Production*, vol. 29, pp. 35-47, 1997.
- [25] S.B. Ayssiwede, A. Dieng, C. Chrysostome, W. Ossebi, J.L. Hornick and A. Missohou, “Digestibility and metabolic utilization and nutritional value of *Leucaena leucocephala* (Lam.) leaves meal incorporated in the diets of indigenous Senegal chickens,” *International Journal of Poultry Science*, vol. 9, pp. 767-776, 2010.
- [26] D.J. McClements and E.A. Decker, “Designing Functional Foods,” Wood head Publishing, Cambridge, UK, 2009.
- [27] S.E. Atawodi, D. Mari, J.C. Atawodi and Y. Yahaya, “Assessment of *Leucaena leucocephala* leaves as feed supplement in laying hens,” *African Journal of Biotechnology*, vol. 7, no. 3, pp. 317-321, 2008.
- [28] S.M.A. Sallam, “Nutritive value assessment of the alternative feed resources by gas production and rumen

fermentation *in vitro*,” *Research Journal of Agriculture and Biological Sciences*, vol. 1, no. 2, pp. 200-209, 2005.

- [29] A.A. Aganga, and S.O. Tshwenyane, “Lucerne, lablab and *Leucaena leucocephala* forages: production and utilization for livestock production,” *Pakistan Journal of Nutrition*, vol. 2, no. 2, pp. 46-53, 2003.
- [30] R.A. Wheeler, W.R. Chaney, K.D. Johnson and L.G. Butler, “*Leucaena* forage analysis using near infrared reflectance spectroscopy,” *Animal Feed Science and Technology*, vol. 64, pp. 1-9, 1996.
- [31] N.N. Maw, S.M. Khin, A. Aung and T.H. Moe, “The nutritive value of some tree foliages locally available in Myanmar,” *Proceedings of an International Conference on International Agricultural Research for Development*, pp. 1-4, 2006.