**Nutritional Characterisation of Leucaena leucocephala Leaves: Insights for Sustainable Livestock Production**

**Abstract**

**Leucaena leucocephala, a drought-resistant leguminous tree, is widely utilized in animal feed due to its high nutritional value. This study evaluates the chemical composition, mineral content, and amino acid profile of Leucaena leucocephala leaves to assess their potential as an alternative protein source for ruminants. The leaves were collected, dried, and ground for proximate analysis, amino acid determination, and mineral estimation. The results revealed that Leucaena leaves have a high crude protein content (23.17% DM), moderate crude fiber (13.67%), and a significant concentration of minerals, particularly calcium (2.19%) and potassium (1.45%). Trace minerals such as iron (337.91 ppm) and boron (64.63 ppm) were also present in notable quantities. Amino acid analysis showed a balanced profile with essential amino acids like lysine, histidine, and methionine, though cysteine and methionine were identified as limiting amino acids. The findings support the potential use of Leucaena leucocephala as a valuable and sustainable protein source in livestock feeding, though attention should be given to the presence of mimosine, a toxic compound. This research highlights the importance of exploring unconventional and local feed resources to reduce feed costs and improve livestock productivity.**

***Keywords:*** *Leucaena leucocephala, alternative protein source, mimosine, unconventional feed, sustainable livestock feeding, forage legume.*

1. **INTRODUCTION**

The primary objective of any livestock industry is to achieve sustainable production in the shortest time frame, ensuring that people have affordable access to animal protein sources (S.B. Ayssiwede *et al*., 2011). However, the rising cost of livestock feed concentrates poses a significant challenge (OECD and Food and Agriculture Organization of the United Nations, 2010). To meet livestock nutritional needs economically, it is crucial to identify and utilize alternative low-cost feed resources (Lekule & Kyvsgaard, 2003). Enhancing the productivity of indigenous livestock is essential for contributing effectively to poverty alleviation and improving food security. This can be achieved by adopting better feeding strategies that incorporate unconventional and locally available feed resources (S.B. Ayssiwede *et al*., 2011). The escalating cost of traditional protein-rich ingredients has driven animal nutritionists to explore alternative protein sources, both vegetable and animal, including insects (AGBO, A.N., 2020). Addressing the shortage of protein ingredients exacerbated by territorial constraints and rising demand requires focused research on such alternatives. Tree fodders have long played a vital role in ruminant diets, traditionally utilized by farmers in many regions. These fodders serve as a reliable resource to bridge feed gaps, providing essential nitrogen, energy, minerals, and vitamins (K.S. Giridhar *et al.,* 2018). Integrating tree fodders into livestock diets offers a sustainable and readily available solution to enhance nutrition and productivity.

Leucaena leucocephala (Lam. de Wit), commonly known as "leucaena," is known by various local names around the world, such as Lamtoro in Indonesia, Koanaole in Hawaii, Subabul in India, and Ipil-ipil in Bangladesh and the Philippines. It is a fast-growing, drought-resistant, tropical leguminous plant widely used in the animal feed industry (M.S. Dorothy *et al*., 2018). Originally native to Mexico, Leucaena leucocephala is now cultivated in many tropical and subtropical regions (Heuze and Tran, 2014). It belongs to the Fabaceae family, Mimosoidae sub-family, and Leucaena genus. The tree features bipinnate leaves 15–20 cm long, lanceolate leaflets, flat brown pods with small seeds, and white flowers (Heuze and Tranc, 2014). This multipurpose tree serves various functions, including use as a windbreaker, ruminant feed, agroforestry species, nitrogen fixer, and source for paper and timber production (Hetrampf and Piedad-Pascual, 2003). The leaves are rich in crude protein, ranging from 15.2% to 34.3% of dry matter (Adedeji *et al*., 2013). They also contain vitamins A and B, carotene, and all 10 essential amino acids (Monoj and Bandyopadhyay, 2007). The leaves offer a nearly complete ruminant feed due to their balance of nutrients and roughage. They can be used for grazing or as fresh or pelleted fodder. Seasonal variations and cutting age impact the chemical composition and digestibility of Leucaena forage (Verdecia et al., 2020). Using Leucaena leucocephala can significantly reduce feed costs while providing balanced nutritional values (Tiamiyu L.O*. et al.,* 2015). It is highly palatable for cattle, though they may need a few days to adjust to grazing it. Amino acids, as the building blocks of protein, are essential for determining the quality and bioavailability of protein-rich ingredients (Robinson and Menghe, 2007). However, the presence of mimosine, a toxic non-protein amino acid, is a significant concern for intensive use of Leucaena leaves. Mimosine is an anti-nutritional factor (ANF) that can inhibit animal growth (S.B. Ayssiwede et al., 2011; Sotolu A.O*. et al*., 2008).

**2. MATERIALS AND METHODS**

Leaves of Leucaena leucocephala were collected from different locations near the College of Veterinary Science in Rajendranagar. The collected leaves were dried separately in hot air oven at $100^{0}$C, ground to pass a 2 mm sieve in Willey mill and saved in polythene bags for further analysis. Proximate analysis, amino acid assessment and mineral estimation were carried out at the Animal Nutrition Lab situated in the College of Veterinary Science at Rajendranagar.

The proximate composition of the Leucaena leucocephala leaves was determined using the standard methods outlined by the Association of Official Analytical Chemists. Dry matter (DM) content was determined by drying the sample at $105^{0}$C in forced air oven till the constant weight. Ash content was measured after igniting sample in a muffle furnace at 550C for 4 hours (h). Crude protein (CP) (N × 6.25) was determined by Kjeldahl method (Anon., 1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were measured using the methods described by Van Soest et al. (1991). The results are presented in Table 1.

The mineral analysis was conducted using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). A 1g portion of the dried powdered sample was transferred into a microwave digestion vessel. To this, 9 mL of concentrated nitric acid (HNO₃) and 1 mL of hydrogen peroxide (H₂O₂) were added. The sample was digested following standardized procedures. After digestion, the vessel was removed and allowed to cool to room temperature. The resulting digestate was filtered through Whatman filter paper No. 42, and the filtrate was quantitatively transferred into a 100 mL volumetric flask. The solution was then diluted to volume with Millipore water. Standard solutions for calibration were prepared by serial dilution of a 1000 µg/g stock standard solution with 1% nitric acid (HNO₃). Sample analyses were performed in triplicates, and the reported data in Table 2 represents the average of the triplicate measurements.

Amino acid analysis was performed on leucaena leaf samples using High-Performance Liquid Chromatography (HPLC). Approximately 0.1 g of the sample was weighed and placed into a closed test tube. To this, 5 mL of 6 N hydrochloric acid (HCl) was added, and the mixture was homogenized using a vortex mixer. The test tube was then purged with nitrogen gas to maintain an inert atmosphere. The sample was incubated in an oven at 110°C for 22 hours. After incubation, the sample was allowed to cool, and the contents were transferred to a 50 mL volumetric flask and diluted to the mark with aquabidest (distilled water). The solution was then filtered through a 0.45 µm filter membrane. A 500 µL aliquot of the filtrate was mixed with 40 µL of acetic acid-borate buffer (AABA) and 460 µL of aquabidest. From this mixture, 10 µL was transferred and reacted with 70 µL of AccQ-Fluor Borate reagent. The resulting solution was homogenized and allowed to stand for one minute before being incubated at 55°C for 10 minutes. Finally, 5 µL of the prepared sample solution was injected into the HPLC column for analysis.

**3. RESULTS AND DISCUSSION:**

**3.1. Chemical composition** :The chemical composition of Leucaena leucocephala leaves is shown in Table 1. The analysis reveals that Leucaena leaves are relatively rich in crude protein (CP), with a content of 23.17% (DM). Similar CP value of 24.9% (DM) have been reported by S.B. Ayssiwede *et al*. (2010). This high CP content indicates that Leucaena leaves, also known as Subabul, have significant potential as a protein source in ruminant diets. The study also found a high dry matter (DM) content of 91.63%, suggesting that Leucaena leaves can store substantial amounts of carbohydrates, proteins, and other structural or metabolic compounds. Dry matter productivity varies depending on soil fertility and rainfall (Anna DeAngelis *et al*., 2021). This DM value is slightly lower than the 92.04% reported by S.B. Ayssiwede *et al.* The analysis also indicated organic matter (OM) and crude fiber (CF) contents of 88.82% and 13.67%, respectively. The CF value is slightly lower than the 14.2% observed by S.B. Ayssiwede *et al.* Additionally, the total ash content of 11.17% suggests a good mineral profile, contributing essential minerals to livestock nutrition. Regarding cell wall components, the study recorded neutral detergent fiber (NDF) and acid detergent fiber (ADF) values of 47.97% and 20.74%, respectively. These values are relatively higher compared to the findings of K.S. Giridhar *et al*., who reported NDF and ADF values of 36.02% and 22.66%, respectively. This higher fiber content further emphasizes the robustness of Leucaena leaves as a forage source for ruminants.

**3.2. Mineral composition:** The mineral composition of Leucaena leucocephala leaves is detailed in Table 2. The macro-mineral analysis indicates high concentrations of calcium (Ca) at 2.19% and potassium (K) at 1.45%. Moderate levels of sulfur (S) at 0.32% and magnesium (Mg) at 0.26% were observed, while phosphorus (P) and chlorine (Cl) were present in lower concentrations, at 0.13% and 0.05%, respectively. In comparison, a study by S.B. Ayssiwede *et al.* reported different macro-mineral values: Ca (1.8% DM), K (1.1% DM), P (0.2% DM), and Na (0.0% DM). Similarly, Anna DeAngelis *et al.* recorded variable concentrations, including Ca (16.0–20.8 g/kg DM), P (2.0–2.4 g/kg DM), Mg (3.4 g/kg DM), and K (17.0 g/kg DM).

Table 3 presents the trace mineral profile of Leucaena leaves. The analysis showed high levels of iron (Fe) at 337.91 ppm and boron (B) at 64.63 ppm. Moderate concentrations were observed for manganese (Mn) at 36.95 ppm, zinc (Zn) at 24.51 ppm, copper (Cu) at 21.81 ppm, and chromium (Cr) at 12.58 ppm. Lower levels were recorded for lithium (Li) at 5.81 ppm, selenium (Se) at 2.78 ppm, and cobalt (Co) at 0.18 ppm. In comparison, Anna DeAngelis et al. reported different trace mineral concentrations, including Fe (907.4 mg/kg DM), Mn (50.9–80.0 mg/kg DM), and Zn (19.2 mg/kg DM). Similarly, D’Mello and Taplin recorded values for Cu (11.4 mg/kg DM), Fe (907.4 mg/kg DM), Zn (19.2 mg/kg DM), and Mn (50.9 mg/kg DM).

Table 4 provides data on heavy metals in Leucaena leaves. The analysis detected lead (Pb) at 4.3 ppm, arsenic (As) at 1.42 ppm, and cadmium (Cd) at 0.07 ppm. These values provide insights into the safety and potential risks associated with using Leucaena as livestock feed.

**3.3. Amino acid composition:** The amino acid composition of Leucaena leucocephala leaves is presented in Table 5. The analysis identified several essential amino acids, including lysine (1.222%), histidine (0.447%), arginine (1.158%), valine (1.141%), methionine (0.354%), threonine (0.86%), and phenylalanine (1.118%). Among the non-essential amino acids detected were aspartic acid, serine (0.826%), glutamic acid (2.047%), proline (1.077%), glycine (1.016%), alanine (1.115%), and cysteine (0.232%). Cysteine and methionine were identified as the limiting amino acids in Leucaena leaves. In comparison, a study by AGBO, A.N. reported different essential amino acid concentrations, including lysine (6.05 g/100g protein), histidine (2.97 g/100g protein), arginine (6.37 g/100g protein), valine (6.02 g/100g protein), methionine (1.77 g/100g protein), isoleucine (5.91 g/100g protein), leucine (9.72 g/100g protein), threonine (5.72 g/100g protein), and phenylalanine (6.66 g/100g protein). The non-essential amino acids in the same study included aspartic acid (9.87 g/100g protein), serine (4.99 g/100g protein), glutamic acid (12.72 g/100g protein), proline (4.47 g/100g protein), glycine (5.91 g/100g protein), alanine (6.30 g/100g protein), tyrosine (5.72 g/100g protein), and cysteine (1.32 g/100g protein). Additionally, an amino acid analysis by Anna De Angelis *et al.* showed varying concentrations, with arginine at 294 mg/g N, cysteine at 88 mg/g N, histidine at 125 mg/g N, isoleucine at 563 mg/g N, leucine at 469 mg/g N, lysine at 313 mg/g N, methionine at 100 mg/g N, phenylalanine at 294 mg/g N, threonine at 231 mg/g N, tyrosine at 263 mg/g N, and valine at 338 mg/g N. These variations highlight the nutritional value of Leucaena leaves, which contain essential and non-essential amino acids beneficial for livestock. However, the lower levels of methionine and cysteine indicate that they are the limiting amino acids in Leucaena protein.

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| **Table 1:**  Proximate analysis and van soest analysis of the leucaena leaves |
| **Parameter**  | **Values (%)** |
| Dry matter  | 91.63 |
| Organic matter  | 88.82 |
| Crude Protein  | 23.17 |
| Crude Fat  | 9.32 |
| Crude Fiber  | 13.67 |
| Total Ash  | 11.17 |
| Nitrogen Free Extract  | 42.65 |
| NDF  | 47.97 |
| ADF  | 20.74 |
| ADL | 17.46 |
| \*Each value is the average of duplicate observations |

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| **Table 2**: Macro Mineral analysis of the leucaena leaves |
| **Mineral** | **Concentration(%)** |
| Calcium  | 2.19 |
| Phosphorous  | 0.13 |
| Sulphur  | 0.32 |
| Magnesium  | 0.26 |
| Potassium  | 1.45 |
| Chlorine  | 0.05 |
| \* Each value is the average of nine observations |

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| **Table 3:** Trace mineral analysis of the leucaena leaves |
| **Mineral** | **Concentration(ppm)** |
| Cu | 21.81 |
| Fe | 337.91 |
| Zn | 24.51 |
| Mn | 36.95 |
| Co | 0.18 |
| Cr | 12.58 |
| Se | 2.78 |
| Ni | 8.55 |
| B | 64.63 |
| Li | 5.81 |
| \* Each value is the average of nine observations |

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| **Table 4:** Toxic mineral analysis of the leucaena leaves |
| **Mineral** |  **Concentration(ppm)**  |
| Pb |  4.3 |
| Cd |  0.07 |
| As |  1.42 |
| \* Each value is the average of nine observations |

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| **Table 5:** Amino acid analysis of leucaena leaves |
| **Parameter** | **Content (% as is)** | **Content (%)\*** |
| Dry matter | 90.64 |  - |
| CP | 24.18 | 23.48 |
| Methionine | 0.354 | 0.344 |
| Cystine | 0.232 | 0.225 |
| Methionine + Cystine | 0.586 | 0.569 |
| Lysine | 1.222 | 1.186 |
| Threonine | 0.86 | 0.835 |
| Arginine | 1.158 | 1.124 |
| Valine | 0.1.141 | 1.108 |
| Histidine | 0.447 | 0.434 |
| Phenylalanine | 1.118 | 1.085 |
| Glycine | 1.016 | 0.986 |
| Serine | 0.826 | 0.802 |
| Proline | 1.077 | 1.046 |
| Alanine | 1.115 | 1.083 |
| Aspartic acid | 2.071 | 2.011 |
| Glutamic acid | 2.047 | 1.987 |
| NH3 | 0.466 | 0.452 |
| Total including NH3 | 15.15 | 14.709 |
| Total without NH3 | 14.684 | 14.256 |
| \* DMS: Figures standardized to a dry matter content of 88%, CP = Crude protein, based on Dumas combustion method (CP factor 6.25). |

**4. Conclusion**

Leucaena leucocephala leaves exhibit promising nutritional qualities, with high crude protein content, essential minerals, and a well-balanced amino acid profile, making them a viable alternative protein source for ruminant livestock. The chemical composition indicates that Leucaena leaves are rich in crude protein (23.17% DM), which is comparable to traditional protein sources used in animal feed. The mineral content, including high levels of calcium and potassium, further supports their potential as a valuable feed ingredient. However, the presence of mimosine, a toxic compound, limits the intensive use of Leucaena leaves without proper management. Despite this, the results suggest that Leucaena can be an effective and sustainable feed resource, particularly in regions where traditional feed costs are high, and alternative feed ingredients are needed. Further research and proper feeding strategies to mitigate the effects of mimosine would enhance its utilization in livestock diets.

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