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Determination of Crude Protein and Amino Acid from *Musa Sapientum L.* Waste as a Protein Source for Animal Feed

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Musa sp. wastes represent a major agro-industrial byproduct with enormous potential to become a sustainable protein source for animal feed, tackling significant food waste and reducing environmental impact. This research aims to address the knowledge gap regarding the *Musa* sp. plant waste by determining the crude protein content through chemical analysis and characterizing its amino acid composition. The Bradford assay method was used to assess the crude protein, with *Musa Sapientum L.* leaves yielding the highest value at 3.220 ± 0.044 mg/mL, followed by peels (1.076 ± 0.011 mg/mL) and stems (0.119 ± 0.004 mg/mL). *Musa sp*. peels, on the other hand, indicated higher values of essential and non-essential amino acids using High-Performance Liquid Chromatography, totaling 45.05 ± 3.43 g/100 g protein and 57.56 ± 4.18 g/100 g protein, respectively. In particular, the essential amino acid lysine was found in the highest quantity in the peel, at 8.05 ± 0.94 g/100 g protein, and glycine was the most abundant non-essential amino acid, at 13.02 ± 1.02 g/100 g protein. Overall, *Musa* sp. leaves exhibit high total protein content, while peels boast a diverse spectrum of essential and non-essential amino acids, including abundant lysine. *Musa* sp. leaves and peels exhibit distinct yet promising protein and amino acid profiles, suggesting their potential for targeted applications in animal feed formulations aimed at improving animal growth and reduced feed costs. Investigating digestibility, anti-nutrients, and optimal inclusion levels will be crucial for unlocking their potential as sustainable and cost-effective feed components.

Keywords: Protein, amino acid, Musa Sp., animal, feed

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Animal feed is a vital component in livestock production, directly influencing animal growth, productivity, health, and welfare. It plays a crucial role in ensuring the availability of high-quality animal-based products such as meat, milk, and eggs. As global demand for animal protein continues to rise, the livestock sector faces increasing pressure to enhance feed efficiency and sustainability. According to the Food and Agriculture Organization (FAO), demand for livestock products is expected to grow by 70% by 2050 to meet the needs of the expanding global population [1].

Protein is one of the most important nutrients in animal feed, serving as the primary source of amino acids essential for tissue development, enzyme synthesis, and immune function. Conventional protein sources such as soybean meal and fishmeal are widely used in commercial feed formulations; however, their rising costs, fluctuating availability, and environmental concerns highlight the urgent need for alternative protein sources [2]. In Malaysia, much of the animal feed is imported, creating a dependency that exposes the industry to global market volatility [3].

Agro-industrial by-products present a promising solution for addressing feed challenges in a sustainable manner. Among these, banana (*Musa sp.*) waste is generated in large quantities and includes peels, pseudo-stems, and leaves. The banana plant is cultivated extensively across tropical regions, including Malaysia, and approximately 30–40% of the harvested bananas are discarded due to cosmetic imperfections or post-harvest spoilage [4]. These residues are often left unused or improperly disposed of, contributing to environmental pollution.

Musa sp. waste contains valuable bioactive compounds such as dietary fiber, polyphenols, antioxidants, and proteins, making it a potential resource for livestock nutrition. Previous studies have shown that banana peels, leaves, and pseudo-stems contain appreciable amounts of crude protein and essential amino acids, which are necessary for animal

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growth and health [5,6]. In particular, lysine, an essential amino acid often limiting in plant-based feeds, has been found in significant quantities in banana peels [7].

Despite its potential, there is limited scientific data on the comprehensive protein and amino acid profile of different *Musa sp*. plant parts, particularly in the Malaysian context. Moreover, understanding the specific nutritional composition of these waste materials is essential to optimize their inclusion in feed formulations without compromising animal health or performance.

Therefore, this study aims to evaluate the crude protein content and amino acid composition of banana (*Musa sapientum L*.) peels, leaves, and stems using established biochemical techniques. The findings are expected to provide insight into the feasibility of utilizing *Musa sp*. waste as a sustainable and cost-effective protein source in animal feed, supporting local feed innovation and reducing reliance on imported materials.

EXPERIMENTAL

Chemicals and Materials

Parts of the *Musa Sapientum L*. plant, such as leaves and stems, were collected from the nearest orchard called Chay's FarmLand located in Kuantan, Pahang. However, the waste of *Musa sp*. peels is collected from the local vendors who are selling fried *Musa sp*. fritters. For chemicals, sodium hydroxide, 3.5% sodium chloride, 95% ethanol, 85% phosphoric acid, Bovine Serum Albumin (Acros Organics), Coomassie Brilliant Blue G-250 (Sigma Aldrich) and acetonitrile were used in this study. All the chemicals involved were analytical grade.

Sample Preparation

Fresh *Musa Sapientum L*. peels, leaves, and stems were meticulously collected from vendors and promptly freeze-dried to preserve their integrity. The freeze-drying process was carried out at -80 °C for 5 days to ensure optimal preservation of the botanical material. Subsequently, the dried components of *Musa* sp. (peels, leaves, and stems) were finely ground using a high-performance grinder with a precise sieve size of 0.75 µm, resulting in a uniformly homogeneous particle size. To maintain the freshness and stability of the powdered *Musa* sp. materials for further experimentation, they were stored in airtight containers at a controlled chiller temperature of +4 °C. This meticulous handling and storage approach aimed to uphold the quality of the botanical samples throughout the course of the subsequent experiments.

Determination of Crude Protein

Protein Extraction

About 0.5 g of dried *Musa sp*. peel was homogenized with 30 mL of 0.1 M sodium hydroxide (NaOH) in 3.5% sodium chloride (NaCl) using MX-S Vortex mixer (DLAB). The homogenates were placed in water bath at 60 °C for 90 min before centrifugation at 4000× g for 30 min. The supernatants were frozen and kept at -20 °C until analyses. The same extraction procedures were repeated with dried *Musa sp*. leaf and stem [8].

Bradford Protein Assay

Bovine serum albumin (BSA) standards (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) were prepared by diluting a BSA stock solution in distilled water. Protein samples were diluted to an appropriate concentration within the BSA standard curve linear range using distilled water. 20 µL of either BSA standards or diluted samples were added to cuvettes containing 980 µL distilled water, followed by the addition of 1000 µL Bradford reagent to each cuvette. After mixing thoroughly by inversion, the cuvettes were incubated at room temperature for 5 min and samples were not incubated for more than 1 h. Absorbance was measured at 595 nm using a spectrophotometer, with a blank distilled water cuvette 43 used to zero the instrument. This process was performed in triplicate for each plant peel, stem, and leaf sample. BSA standard solutions were used to construct a standard curve by plotting absorbance against known BSA concentration. Sample protein concentrations were calculated by comparing their average absorbance values against the standard curve [9].

Table 1. Crude protein content in peels, leaves, and stems of *Musa Sapientum L*.

Sample	**Crude Protein (mg/mL)**		
	Peels	**Leaves**	**Stem**
Musa sp.	1.076 ± 0.011	3.220 ± 0.044	0.119 ± 0.004

Determination of Amino Acids by High-Performance Liquid Chromatography (HPLC)

A reversed-phase chromatography (RPC) was employed as it is the most popular technique since it can handle the broadest variety of sample types. For all chromatographic separations, an Agilent Technologies model 1260 Infinity semi-prep HPLC System was used, including the solvent and sample module, integrated vacuum degasser and column oven, and MS detector. The derivatized samples was diluted to 1 mL with acetic acid after cooling to room temperature, and the amino acid contents were determined. The analytes were separated using C18 columns called octadecyl in the stationary phase and ACN from the mobile phase which was programmed by the autosampler. Before injection, all calibrants and samples were filtered through 0.45 µm filters to remove any small particles. The analysis time was conducted for 25 min with a flow rate of 0.400 mL/min, pressure 2100 psi, oven temperature of 35 °C, and 5 µL of injection volume [10].

RESULTS AND DISCUSSION

Crude Protein

The Bradford assay is an extremely sensitive and simple technique that is able to detect a wide range of proteins, including amounts as small as 1–20 µg [11]. Through this study, the Bradford assay is employed to measure the crude protein content of *Musa* sp. waste, aiming to evaluate its potential as a sustainable protein source for animal feed. Table 1 shows the crude protein content obtained from peels, leaves, and stems of *Musa* sp.

The analysis in Table 1 reveals that *Musa* sp. leaves showed the highest value at 3.220 ± 0.044 mg/mL compared to peels (0.1076 ± 0.011 mg/mL) and stems (0.110 ± 0.032 mg/mL). This suggests that the leaves are the primary site of biosynthesis for this compound, as they contain significantly higher levels of the compound compared to the peels and stems.

These practical results partially mirror theoretical values, with Musa sp. leaves exhibiting the highest protein content (11.25 ± 1.18%) compared to both peels (1.95 ± 0.14%) and stems (7.25 ± 0.66%) [12]. This suggests a targeted localization of protein production within the plant. However, it's important to acknowledge that the analytical approach in this study differed from literature as the 45 measured protein content was in percentage. These discrepancies in units could partially explain the variations observed between the results and theoretical values. However, when exposed to air, Musa sp. peel deteriorates rapidly, resulting in animal rejection and food waste [13]. *Musa* sp. peel meal can provide nutrients but becomes less suitable in poultry diets at inclusion rates over 15% as higher Musa sp. peel concentrations negatively impact feed efficiency and growth rate in broiler chickens when compared to corn and soy feed [14]. Even though, *Musa* sp. peel is a plant-based feed, it becomes less productive for the chickens at very high dietary concentrations instead of conventional ingredients. However, according to the study of Amarnath, *Musa* sp. stems and leaves are a good source of ruminant feed [15]. In addition, Marie-Magdeleine et al., (2009) reported *Musa* sp. leaves as good feed because of their non-significant differences in crude protein and fibre content compared with good quality feed, which can be used to sustain animals during seasonal feed shortages [16]. Overall, *Musa* sp. leaves may have suitable dietary protein levels to partially replace protein ingredients like soybean meal in plant-based animal feed formulations.

Amino Acid Composition

Chromatography techniques, particularly high-performance liquid chromatography (HPLC), are commonly employed for quantitative analysis due to their ability to separate amino acids in dietary samples [17]. Table 2 shows the list of presented amino acids from *Musa sp.* peels, leaves, and stems.

The analysis of *Musa sp.* waste in Table 2 revealed that the peel contains significantly higher quantities of non-essential amino acids, with a total of 57.56 ± 4.18 g/100 g protein, in comparison to the leaves and stems. Among the essential amino acids, the peel was found to have the highest content, totalling 45.05 ± 3.43 g/100 g protein, which suggests a robust potential for nutritional exploitation in dietary applications. The leaf's amino acid profile was characterized by a relatively lowest content of both essential and non-essential amino acids, with totals of 11.24 ± 1.07 g/100 g protein and 16.6 ± 2.30 g/100 g protein, respectively. The stem's composition of essential amino acids amounted to 34.52 ± 2.14 g/100 g protein, indicating a valuable source of these vital nutrients. Glycine was the most abundant non-essential amino acid in the *Musa sp*. peel, registering a concentration of 13.02 ± 1.02 g/100 g protein. Comparatively, the essential amino acid lysine was present in the highest amount in the peel, at 8.05 ± 0.94 g/100 g protein, which is essential for human nutrition. The findings suggest that *Musa sp*. waste, particularly the peel, is a rich source of amino acids and could be utilized in developing value-added products.

Table 2. Amino acid composition of *Musa Sapientum L.* waste (peels, leaves and stems).

Amino acids	**Content (g/ 100 g protein)**		
	Peels	**Leaves**	**Stems**
Valine	2.52 ± 0.2	0.48 ± 0.01	3.04 ± 0.09
Lysine	8.05 ± 0.94	1.22 ± 0.08	5.42 ± 0.82
Leucine	7.81 ± 0.06	2.13 ± 0.07	6.31 ± 0.52
Isoleucine	7.05 ± 1.04	2.13 ± 0.45	6.10 ± 0.98
Phenylalanine	5.86 ± 0.07	1.49 ± 0.06	3.21 ± 0.13
Threonine	3.14 ± 0.03	0.98 ± 0.04	2.70 ± 0.02
Histidine	4.16 ± 0.01	0.75 ± 0.00	4.33 ± 0.16
Methionine	3.45 ± 0.03	0.82 ± 0.01	2.20 ± 0.05
Tryptophan	3.01 ± 0.00	1.24 ± 0.02	1.21 ± 0.02
Total essential amino acids	**45.05 ± 3.43**	**11.24 ±1.07**	**34.52 ± 2.14**
Arginine	10.21 ± 1.05	3.87 ± 0.58	8.33 ± 1.70
Aspartic acid	9.06 ± 0.05	2.15 ± 0.07	6.42 ± 1.09
Glutamic acid	5.31 ± 0.01	1.40 ± 0.05	3.44 ± 0.05
Serine	4.59 ± 0.04	0.43 ± 0.01	2.34 ± 0.06
Glycine	13.02 ± 1.02	5.30 ± 1.53	13.02 ± 1.02
Alanine	4.85 ± 0.04	0.89 ± 0.01	4.85 ± 0.04
Cysteine	5.03 ± 0.03	1.14 ± 0.01	4.11 ± 0.08
Tyrosine	5.50 ± 0.03	1.42 ± 0.07	2.83 ± 0.14
Total non-essential amino acids	**57.56 ± 4.18**	**16.6 ± 2.30**	**45.34 ± 3.71**

Musa sp. peel shows some similarity in its highest compositional data on amino acids, revealing that lysine is the most abundant essential amino acid at 6.71 ± 0.06 g/100 g of protein,while glycine has the highest level of any non-essential amino acid at 13.02± 0.82 g/100 g of protein [18]. Despite this, the importance of these amino acids in animal feed remains undeniable. Lysine deficiency, for instance, negatively impacts animal health and growth performance [19]. Conversely, studies show that supplementing pig feed with 0.52% glycine enables growing pigs to achieve similar average daily weight gain and feed efficiency as pigs fed a normal protein diet [20]. Next, the literature reports that the essential amino acid leucine is most abundant in *Musa sp*. stems at 27.19 ± 0.02 mg/g of protein, accounting for around 41% of total essential amino acids while tryptophan has the lowest concentration at 3.70 ± 0.03 mg/g of protein, making up 34% of essential amino acids in *Musa sp*. stems [21]. Additional research shows that animal nutritionists often do not specify a minimum dietary requirement for leucine. This is because leucine levels tend to greatly surpass animal needs, at least in typical US diets. Many common ingredients in US animal feeds, especially corn and corn products, contain disproportionately high ratios of leucine compared to other branched-chain amino acids like valine and isoleucine [22].

However, there are some differences between literature and practical results. The non-essential amino acid glycine was reported at slightly higher levels in *Musa sp*. stems (14.44 ± 0.06 mg/g of protein) compared to practical findings, while glutamic acid had the highest concentration at 63.75 ± 0.05 mg/g of protein-based on literature review. These variations may be attributed to the use of a specific *Musa sp*. cultivar (*Musa sp. cv. Nanjangud rasa bale*) in the study, which can impact amino acid profiles. Additionally, despite limited evidence in existing publications, this practical work verified relatively low total amino acid content in *Musa sp*. leaves compared to peels and stems. Although *Musa* sp. leaves recorded the highest crude protein levels, most of these proteins are structural or photosynthetic, such as chloroplast and RuBisCO proteins, which are tightly bound within fibre-rich tissues and not easily hydrolysed during amino acid extraction.

In contrast, banana peel contains lower total protein but a much higher proportion of soluble or hydrolysable proteins and free amino acids that are readily detected by HPLC. The peel also acts as a metabolically active storage tissue, accumulating free amino acids such as lysine and glycine during fruit development and ripening. Additionally, the high fibre and phenolic content of leaves may hinder protein release and derivatization efficiency, resulting in lower apparent amino acid values. This unexpected result indicates a need for more research on the nutritional composition of *Musa sp*. leaves, which may be higher in fibre or total phenolics instead of amino acids [5]. In a nutshell, all three *Musa sp.* parts contain useful amino acid profiles, especially lysine and leucine, indicating potential for animal feed supplementation if integrated as part of a balanced diet. Differences among plant parts and cultivars underscore the need for additional feed trials to develop optimal application strategies.

CONCLUSION

Crude protein and amino acids were successfully determined in *Musa Sapientum L.* using the Bradford assay method and HPLC. *Musa sp*. leaves hold the highest concentration of the analyzed compound, with a significantly greater level compared to both peels and stems. This finding suggests that leaves are the main site where this compound is produced in the plant, as their concentration is much higher than in other parts. *Musa sp*. waste, especially the peel, emerges as a powerful source of diverse amino acids, exceeding both leaves and stems in content. Its significant levels of both essential and non-essential amino acids, including lysine and glycine, highlight its tremendous potential for nutritional applications in food and feed development. With optimization, *Musa sp*. wastes have the potential to become alternative feed ingredients, though factors like digestibility and processing require consideration when formulating cost-effective, environmentally conscious animal feeds.

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