International Journal of Agriculture and Biosciences 2025 14(5): 1005-1011.
https://doi.org/10.47278/journal.ijab/2025.116

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

An Alternative Forage for Animal Feed: Nutritional Quality and *in Vitro* Digestibility of Cassava Leaves (*Manihot utilissima*) Soaked in Bamboo Activated Charcoal

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ABSTRACT

This study aimed to analyze and evaluate the nutritional quality and *in vitro* digestibility of cassava leaves (*Manihot utilissima*) using bamboo activated charcoal soaking. The research employed an experimental method based on a completely randomized design, which included four treatments and six replications. The soaking process lasted for 36 hours, with treatments consisting of 0% bamboo activated charcoal (T1), 2% bamboo activated charcoal (T2), 4% bamboo activated charcoal (T3), and 6% bamboo activated charcoal (T4). The parameters observed in this study were proximate analysis (moisture content, dry matter, crude protein, ether extract, crude fiber, ash, nitrogen-free extract, and total digestible nutrients), van Soest analysis (acid detergent fiber, neutral detergent fiber, cellulose, and lignin), and *in vitro* digestibility (dry matter digestibility and organic matter digestibility). Data were analyzed using analysis of variance (ANOVA). When significant differences were observed among treatments, Duncan's multiple range test (DMRT) was applied for post hoc comparisons using SPSS version 24. The results indicated that the level of bamboo activated charcoal had a significant effect (P<0.05) on the nutritional quality and digestibility of cassava leaves. Additionally, the appropriate use of bamboo activated charcoal is crucial to prevent nutrient leaching and maintain feed quality. This study concludes that a 2% level of bamboo activated charcoal can effectively enhance nutritional quality and *in-vitro* digestibility, making it a viable alternative for livestock forage.

Keywords: Bamboo activated charcoal, Cassava leaves, *In vitro* digestibility, Nutritional quality.

Article History
Article # 25-247
Received: 06-May-25
Revised: 15-Jul-25
Accepted: 17-Jul-25
Online First: 28-Jul-25

INTRODUCTION

The current availability of high-quality forage feed is limited due to the decreasing land area designated for livestock farming, including for dual-purpose livestock like Etawa crossbred goats (Prasetyo, 2019). A deficiency in forage quality or quantity leads to a significant decline in milk production, causing these goats to be primarily used for meat in Indonesia (Laya & Ilham, 2019). However, these goats can play a vital role in meeting the national milk demand (Arief et al., 2020). To address this challenge, it is crucial to explore alternative sources of forage feed to enhance milk production in Etawa crossbred goats. One promising option is cassava leaves, which are typically not consumed by humans and are considered a by-product of plantations. Despite this, they have potential as a nutritious feed source for goats, offering protein content, and acting as an anthelmintic for ruminants (Wanapat and Khampa, 2006); as an probiotic (Samedi dan Charles, 2019), and as an inhibitor of mastitis in dairy cows (Cai et al., 2024).

While cassava leaves are often praised for their nutritional benefits, it is crucial to acknowledge that they also contain significant amounts of anti-nutritional substances, particularly hydrocyanic acid (HCN) and tannins (Kiyothong & Wanapat, 2004; Duong et al., 2005; Soto-Blanco & Górniak, 2010). These compounds can pose health risks if not properly managed, making it essential to handle and prepare cassava leaves with care. Siska et al. (2024) stated that soaking cassava leaves in bamboo activated charcoal at concentrations of 2, 4, and 6% for 36 hours can effectively reduce levels of HCN and tannins to within acceptable consumption limits. However, further

Cite this Article as: Siska I, Ako A, Natsir A and Utamy RF, 2025. An alternative forage for animal feed: nutritional quality and *in vitro* digestibility of cassava leaves (*Manihot utilissima*) soaked in bamboo activated charcoal. International Journal of Agriculture and Biosciences 14(5): 1005-1011. https://doi.org/10.47278/journal.ijab/2025.116

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research is necessary to determine whether this reduction in HCN and tannin levels also affects the nutritional quality and digestibility of the treated cassava leaves. Consequently, it is essential to carefully control the concentration of activated charcoal to prevent nutrient leaching and the potential decline in feed quality or content needed for livestock.

Activated charcoal has emerged as a captivating subject of research, owing to its affordability and a wide array of remarkable benefits across diverse sectors (Arsad & Hamdi, 2010). From enhancing agricultural productivity and improving animal husbandry practices to promoting health and safety in industrial applications and culinary uses, its potential is vast and transformative (Gerlach & Schmidt, 2012; Wilson, 2016; Kammann et al., 2017). Utilization of activated charcoal's potential offers an exciting opportunity for advancement and innovation across a wide range of industries (Isa et al., 2016). Activated charcoal can be used as feed additives and an antibiotic substitute to deactivate toxins, improve animal health (particularly the digestive tract), reduce or eliminate the negative effects of anti-nutritional factors such as tannins, increase nutrient absorption (especially protein), and enhance feed digestibility and efficiency (Gerlach and Schmidt, 2012; Kammann et al., 2017; Qomariyah et al., 2019; Schmidt et al., 2019; Jimoh et al., 2020; Hassan and Carr, 2021; Huang et al., 2021; Qomariyah et al., 2023).

According to Jamarun et al. (2020), there are three categories of forage based on CP and TDN content, namely low-quality forage (CP <4%, TDN> 40%), medium-quality forage (CP 5–10%, TDN 40–50%), and high-quality forage (CP> 10%, TDN> 50%). In light of this, a study was conducted to evaluate the impact of varying levels of bamboo activated charcoal on the nutritional quality and *in-vitro* digestibility of cassava leaves, which are being explored as an alternative forage option.

MATERIALS & METHODS

Experimental design and treatment protocol

This study used an experimental method using a completely randomized design (CRD), consisting of 4 treatments and 6 replications. The soaking technique was applied for 36 hours, and treatments in this study were as follows:

T1: 0% bamboo activated charcoal
T2: 2% bamboo activated charcoal
T3: 4% bamboo activated charcoal
T4: 6% bamboo activated charcoal

Sample preparation

The stages carried out in this study included sample preparation (Siska et al., 2024) as shown in Fig. 1, followed by proximate analysis, van Soest analysis, and *in-vitro* analysis.

The parameters observed in this study were:

1. Proximate Analysis: Proximate analysis was conducted using the AOAC (2023), method and included moisture content (MC), dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE), and total digestible nutrients (TDN).
2. Van Soest Analysis: Van Soest analysis was performed using the method van-Soest, (1965) and included acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, and lignin.
3. *In vitro* digestibility Analysis: *In vitro* digestibility analysis was conducted using the method by Tilley and Terry (1963) and included dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD).

Statistical analysis

The data were analyzed using analysis of variance (ANOVA). When significant effects were found among the treatments, analysis was continued with Duncan's Multiple Range Test (DMRT) using SPSS version 24.

RESULTS AND DISCUSSION

Nutritional quality

The nutritional quality of cassava leaves soaked with bamboo activated charcoal is presented in Table 1.

The analysis of variance results on the proximate analysis indicated that soaking cassava leaves in bamboo activated charcoal had a significant impact ($P<0.05$) on the nutritional quality of the leaves. The follow-up DMRT test revealed that treatment T2 showed significant differences, resulting in increased CP, NFE, and TDN, while decreasing CF compared to treatments T1, T3, and T4. Additionally, the analysis of variance based on the Van Soest method demonstrated a significant effect ($P<0.05$) on the contents

Fig. 1: Sample preparation stages include the collection of cassava leaves, separation, chopping, solution preparation, soaking, washing, and drying.

Table 1: Nutritional quality of cassava leaves soaked with bamboo activated charcoal

Parameter	Treatment				SE	P-value
	T1	T2	T3	T4		
Proximate analysis (%)						
Dry matter (DM)	91.948^{b}	93.320^{a}	93.332^{a}	93.417^{a}	0.139	0.000
Moisture content (MC)	8.052^{b}	6.680^{a}	6.668^{a}	6.583^{a}	0.138	0.000
Crude protein (CP)	30.560^{c}	32.885^{a}	31.810^{b}	31.208^{bc}	0.229	0.000
Ether extract (EE)	5.923^{a}	4.850^{b}	4.878^{b}	4.758^{b}	0.138	0.002
Crude fiber (CF)	18.488^{c}	13.915^{a}	17.140^{b}	18.167^{c}	0.396	0.000
Ash	4.180^{a}	4.158^{a}	4.315^{a}	4.985^{b}	0.079	0.000
Nitrogen-free extract (NFE)	40.848^{b}	44.192^{a}	41.857^{b}	40.875^{b}	0.380	0.001
Total digestible nutrients (TDN)	72.198^{b}	77.028^{a}	73.255^{b}	70.740^{c}	0.520	0.000
Van Soest analysis (%)						
Acid detergent fiber (ADF)	28.567^{c}	18.887^{a}	25.885^{b}	26.933^{c}	3.981	0.000
Neutral detergent fiber (NDF)	46.987^{c}	21.052^{a}	42.675^{b}	43.023^{b}	2.126	0.000
Cellulose	18.252^{c}	11.545^{a}	14.302^{b}	16.625^{c}	0.610	0.000
Lignin	14.398^{c}	7.523^{a}	11.423^{b}	14.383^{c}	0.618	0.000

Note: Superscripts with numbers followed by different letters in the same row indicate significant differences (P=0.05). **T1**: 0% bamboo activated charcoal; **T2**: 2% bamboo activated charcoal; **T3**: 4% bamboo activated charcoal; **T4**: 6% bamboo activated charcoal.

of ADF, NDF, cellulose, and lignin in cassava leaves. The results from the DMRT post-hoc test indicated that the ADF, NDF, cellulose, and lignin contents from T2 were significantly different from those in T1, T3, and T4. Treatment T3 also exhibited significant differences from T1, T2, and T4; however, T1 did not show a significant difference from T4, while the NDF levels in T3 and T4 were not significantly different.

The observed increase in DM and CP levels in T2, T3, and T4 can be attributed to the osmosis-diffusion process involving the substances present in bamboo activated charcoal and cassava leaves. It has been noted that soaking initiates an osmosis process in the material, facilitating the entry of free water and dissolved substances into the cassava leaves. Among the various substances found in bamboo activated charcoal are nitrogen (N) and essential minerals, including carbon, calcium, phosphorus, sodium, potassium, magnesium, manganese, iron, and zinc (Kartika and Nisa, 2015). Widodo et al. (2019) indicated that increasing the nitrogen content in forage enhances both the total weight and DM yield. Nitrogen is essential as it positively influences DM yield, CP concentration, and the digestibility of forage (Utamy et al., 2021). Activated charcoal, notably, has a nitrogen content of around 3008.04mgkg^{-1} (Ayankoso et al., 2023), which can contribute to these nutritional benefits. The relationship between DM and moisture content (MC) is inverse; as DM increases, MC tends to decrease. This is supported by the findings of Lambebo and Deme (2022), who demonstrated that a decrease in DM is associated with an increase in MC, and vice versa. In this study, the MC and DM of cassava leaves were observed to be within acceptable ranges. Trisyulianti et al. (2003) suggested that feed MC should be maintained below 14% to inhibit the growth of fungi and microbes. Moreover, nitrogen plays a significant role in influencing CP content. According to Widodo et al. (2019), an elevated nitrogen content in leaves correlates with higher CP levels in the feed. Jamarun et al. (2020) further classify feed materials with a CP content exceeding 10% as high-protein feeds, underscoring the nutritional potential of cassava leaves when supplemented with activated charcoal.

The CP content of cassava leaves in treatments T3 and T4 decreased, primarily due to the nature of activated charcoal as an adsorbent. Excessive concentrations of activated charcoal can absorb beneficial substances for livestock, such as protein. Ohanaka et al. (2021) indicated that overusing activated charcoal can disrupt the compounds present in the material, as its adsorptive properties may capture components that are meant to remain. Furthermore, this phenomenon is influenced by the differences in characteristics and particle sizes between protein and bamboo activated charcoal. Since CP is partially water-soluble and has smaller particle sizes, typically ranging from 10–17m^{2} to 10–12m^{2} (Ripple dan Dimitrova, 2012; Amin et al., 2014), it is more susceptible to being adsorbed by bamboo activated charcoal at higher concentrations, which has pore sizes ranging from 0.074-0.076m^{2} (Hartati et al., 2016; Manurung et al., 2019).

The study found that the contents of CF and EE in cassava leaves were significantly influenced by the soaking process. Yanuartono et al. (2019) demonstrated that when cassava leaves were soaked, water penetrated the cell wall structure, compromising the integrity of cellulose and hemicellulose, which led to a reduction in CF levels. In addition, soaking leads to fat oxidation and affects EE content (Latief et al., 2023). Interestingly, in treatments T3 and T4, both CF and EE levels were observed to increase. This can be attributed to the antioxidant properties of activated charcoal, which help mitigate oxidative damage caused by water. Qomariyah et al. (2019), demonstrated that activated charcoal possesses antibacterial, antioxidant, and antifungal properties, primarily due to its polyphenolic compounds, which can inhibit oxidative damage. Moreover, the components of bamboo activated charcoal can contribute in maintaining the structural stability of plant materials, thereby indirectly influencing the levels of CF and EE. Additionally, the nutritional quality of feed materials is also impacted by the stabilization of structure and reduction in moisture content, as evaluated by McDonald et al. (2018). It's worth noting that, according to Jamarun et al. (2020), a minimum CF requirement of 13% in feed is necessary for livestock, while Palmer et al. (2000) highlighted an EE requirement of at least 5% for ruminants. Excessively high EE levels could disrupt digestion and affect mammary gland function in dairy animals, potentially leading to diarrhoea (Surbakti et al., 2022).

The mineral content in activated charcoal has been found to increase the ash content of feed. A high ash content is indicative of a higher mineral content, which can support growth and development while facilitating metabolic processes in an animal's body (Lambebo & Deme, 2022). Ayankoso et al. (2023), stated that activated charcoal contains several mineral elements such as carbon, calcium, phosphorus, sodium, potassium, magnesium, manganese, iron, and zinc. Additionally, bamboo activated charcoal is noted for having approximately four times the void space, three times the mineral content, and a fourfold improvement in absorption rate compared to standard charcoal (Chu et al., 2013). It is important to note that the maximum ash content for ruminant feed is set at 12%, as an excessive ash content can negatively impact the quality of feed ingredients (Jamarun et al., 2020). Therefore, analysing the crude protein, ash, crude fiber, and ether extract content in T3 and T4 is crucial to determining the optimal level of activated charcoal for feed processing, ensuring that it does not adversely affect the ingredients.

The nutritive value of cassava leaves, as indicated by their NFE and TDN, is influenced by several other nutrient components. Specifically, NFE is determined by the levels of DM, CP, CF, EE, and ash content (Adni et al., 2021). TDN, on the other hand, is influenced by the amounts of CP, EE, CF, and NFE. Research indicates that other components significantly influence the NFE content of a feed material, as it is calculated by subtracting the percentages of CP, EE, CF, and ash from the percentage of DM (Wanapat, 2002; Artanti et al., 2019). The findings of this study suggest that cassava leaves can be classified as high-quality forage, as they exhibit a TDN value greater than 50%, which aligns with established criteria for high-quality forage demonstrated by Jamarun et al. (2020).

The decrease in CF within feed materials is associated with a reduction in the levels of ADF, NDF, cellulose, and lignin. ADF and NDF are known to limit digestibility and are particularly challenging for rumen microbes to degrade. As the CF content increases, the breakdown time for feed in the rumen lengthens due to the complex structure of fibrous materials. The reduction in NDF can be attributed to the breakdown of lignohemicellulose and lignocellulose bonds, as suggested by Tallan et al. (2021), alongside a decrease in CF as noted by Setiyawan dan Thiasari (2016). According to the National Research Council (NRC, 2000), the minimum acceptable levels of ADF and NDF in feed are 19% and 21%, respectively. Surbakti et al. (2023) further indicated that the maximum permissible levels are 45% for ADF and 60% for NDF. These components play a vital role in the digestive system as they contribute to energy sources for livestock. Additionally, Adni et al. (2021) pointed out that a reduction in lignin content often accompanies a decrease in CF, thus facilitating more effective digestion by rumen microbes. The lignin content for livestock has been reported to be around 7% (Van Soest, 1965). This balance is essential to ensure optimal feed digestibility and overall animal health.

***In-Vitro* Digestibility**

The *in vitro* digestibility of cassava leaves soaked with bamboo activated charcoal is presented in Table 2.

The analysis of variance results indicated that soaking cassava leaves in bamboo activated charcoal had a significant effect ($P<0.05$) on both the *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of the cassava leaves. The subsequent DMRT post-hoc test revealed that the IVDMD and IVOMD in treatment T2 were significantly higher than those in treatments T1, T3, and T4. Additionally, treatment T4 exhibited significantly lower values, while T3 did not show a significant difference compared to T1 and T4.

Soaking cassava leaves with bamboo activated charcoal can enhance the *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) observed at T2. Svihus (2014) stated that soaking can increase the digestibility of protein, fat, and ash and increase the energy that can be utilized by livestock. The addition of activated charcoal to the feed significantly increased the IVDMD, IVOMD, and CP of the feed (Van 2006). However, at T3 and T4, with 4 and 6% levels of bamboo activated charcoal, a decrease in IVDMD and IVOMD was noted. This underscores the importance of carefully controlling the concentration of activated charcoal to prevent nutrient leaching and preserve the quality and content of the feed (Adejoro et al., 2018; Ribeiro et al., 2020). Regression analysis of bamboo activated charcoal content against IVDMD and IVOMD revealed a quadratic relationship (Fig. 2). The increase in IVDMD and IVOMD at T2 can be attributed to higher CP alongside a reduction in EE in the treatment. Correlation analysis showed a positive linear relationship between IVDMD and IVOMD with CP, and a negative linear correlation with EE (Fig. 3). According to Ginting (2005), higher values of IVDMD and IVOMD are significantly influenced by the proportions of protein, which serves as a nitrogen source for rumen microbes, carbohydrates that provide a carbon skeleton for rumen microbial protein synthesis, and energy for the host livestock. Additionally Wajizah *et al.* (2015) demonstrated that the digestibility of feed in the rumen is affected by its chemical composition, especially the content of CF and CP which facilitate feed digestibility during fermentation. High dry matter digestibility promotes increased microbial protein synthesis, particularly in terms of crude protein nutrients and soluble carbohydrates.

Table 2: *In vitro* digestibility of cassava leaves soaked with bamboo activated charcoal

Parameter	Treatments				SE	P-value
	T1	T2	T3	T4		
Dry matter digestibility (IVDMD)	64.892^{b}	70.105^{a}	63.630^{bc}	60.975^{c}	0.819	0.000
Organic matter digestibility (IVOMD)	63.789^{b}	67.538^{a}	62.360^{bc}	59.883^{c}	0.736	0.000

Note: Superscripts with numbers followed by different letters in the same row indicate significant differences (P=0.05). **T1**: 0% bamboo activated charcoal; **T2**: 2% bamboo activated charcoal; **T3**: 4% bamboo activated charcoal; **T4**: 6% bamboo activated charcoal.

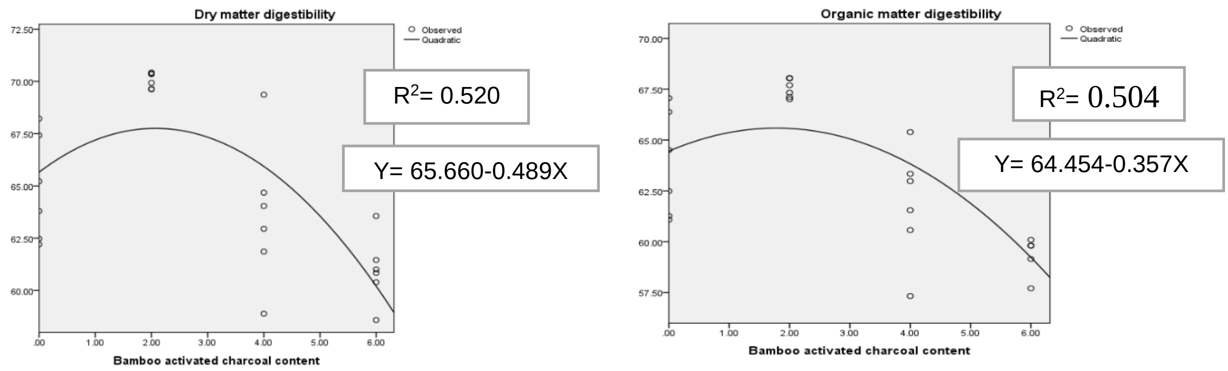

Fig. 2: Correlation of Bamboo activated charcoal (%) with IVDMD and IVOMD (%).

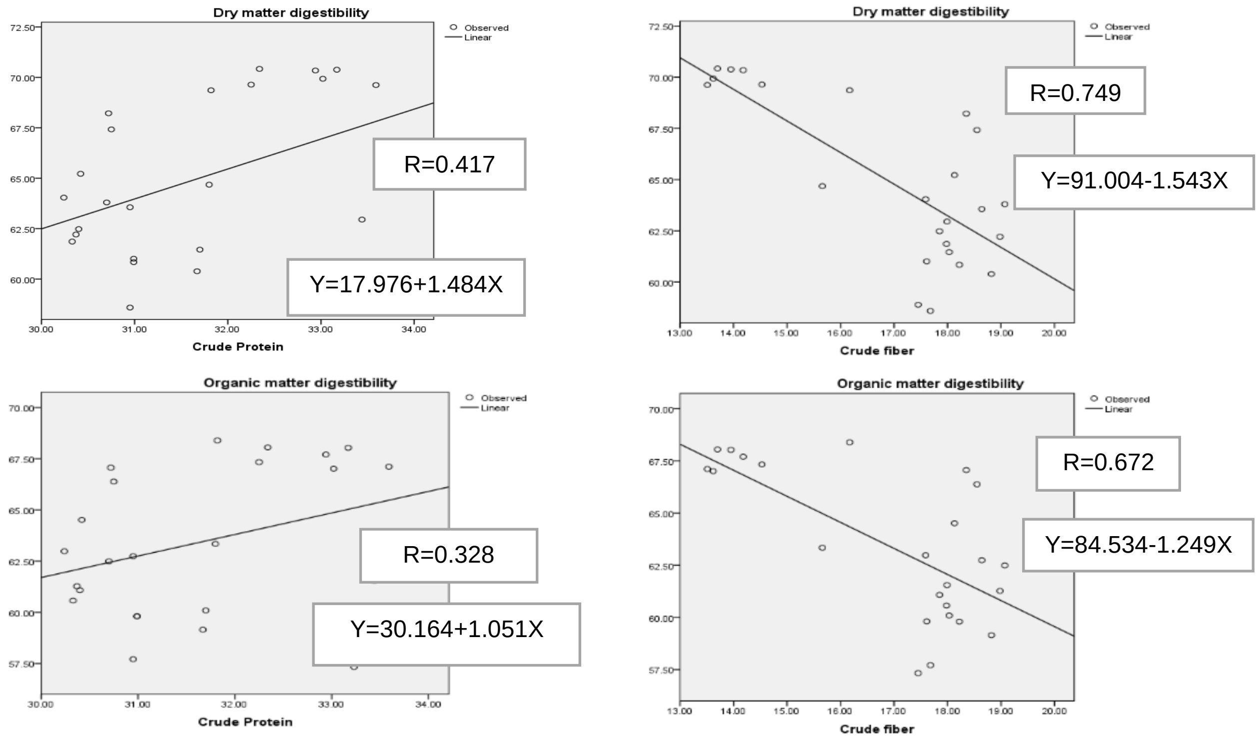

Fig. 3: Correlation of % CP, CF with IVDMD and IVOMD.

Conclusion

The study's that in the treatment with 2% bamboo activated charcoal (T2), there is an increase in DM, CP, NFE, TDN, IVDMD, and IVOMD of cassava leaves, as well as a decrease in MC, CF, EE, ADF, NDF, cellulose, and lignin. Meanwhile, treatments with 4% and 6% bamboo activated charcoal display reduced nutritional quality and digestibility. Additionally, the use of bamboo activated charcoal in the proper amount is essential to prevent loss of nutrients and maintain feed quality. In conclusion, 2% bamboo activated charcoal can effectively enhance nutritional quality and in vitro digestibility, making it a viable alternative for livestock forage.

DECLARATIONS

Funding: Funding of this research is that the 2% bamboo activated charcoal content and 36 hours soaking time are practical and efficient in improving the nutritional quality and digestibility of cassava leaves *in vitro*.

Acknowledgment: The author would like to thank the Indonesian Education Scholarship (BPI), the Center for Higher Education Funding and Assessment (PPAPT), and the Indonesia Endowment Fund for Education (LPDP Indonesia). Ministry of Higher Education, Science, and Technology of the Republic of Indonesia.

Conflict of Interest: The authors have declared no conflict of interest.

Data Availability: The data supporting the findings of this study are presented in this article. There is no other supporting data.

Ethical Statement: Ethical permission and formal approval were granted by the Research Ethics Committee of Hasanuddin University, Hasanuddin University Teaching Hospital (RSPTN) and dr. Wahidin Sudirohusodo Central General Hospi tal (RSUP) Makassar, Indonesia, with approval no. 87/ UN4.6.4.5.31/PP36/2024.

Author's Contribution: Imelda Siska: Conducting the research, data collection, and laboratory work. Ambo Ako: Responsible for research design, corresponding author, and supervision of the research. Asmuddin Natsir: Research design, and supervision of the research. Renny Fatmyah Utamy: Research design, and supervision of the research.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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