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Enhancing nutritional value of cassava cobs through *Aspergillus niger* fermentation: A sustainable approach for animal feed

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ABSTRACT

Cassava cobs, a by-product of cassava processing, have the potential to be utilized as a sustainable alternative feed source for livestock. However, their low protein content and high fiber composition limit their direct application. This study investigated the effect of solid-state fermentation using *Aspergillus niger* on the nutritional profile of cassava cobs. Cassava cobs were incubated with *A. niger* at concentrations of 0, 2.5 and 5% and fermented for 0, 2, and 4 days. The fermented products were analyzed for measurement of proximate composition, fiber fractions, digestible crude protein, and digestibility. The data were analysed using factorial design. The results showed that fermentation with *A. niger* significantly improved the nutritional value of cassava cobs. Fiber content decreased from 32.57% to 25.42%, while digestible crude protein increased from 41.24% to 51.00% at 5% inoculum and 4 days of fermentation. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were reduced, with a concomitant increase in their digestibility. This study demonstrates that solid-state fermentation with *A. niger* as a viable method to enhance the nutritional quality of cassava cobs, supporting their use a functional feed ingredient for sustainable livestock production.

Introduction

Feed is a crucial factor of animal health and productivity (Mironova *et al*., 2021). In the recent years, the livestock industry has encountered significant challenges regarding shortage of quality feed availability. Addressing this issue require the integration of both conventional and novel strategies approaches, including the fermentation technique enhancing feed functionality through modulation of the gut microbiota.

To promote sustainable and cost-effective livestock feed, agro-industrial by-products such as cassava pulp, banana peel meals, and rice bran (Sugiharto *et al*., 2018), corn cobs, rice straw, sugarcane bagasse, and palm kernel cake (Blandino *et al*., 2016; Sadh *et al*., 2018) have been investigated. The low nutritional value of these feeds makes them difficult for animals to digest (Blandino *et al*., 2016). Among these by-products, cassava cobs an underutilized residue (*Manihot esculenta*) and its processing has recently gained attention. Despite its abundance, the application in animal feed is limited due to its low protein and high fiber content (Su and Chen, 2020). Cassava cobs have high carbohydrate and lignocellulosic fiber content, mostly cellulose, hemicellulose, and lignin (Akaracharanya *et al*., 2011; Chirinang and Oonsivilai, 2018), and minor protein, pectin, lipids, and calcium contents. However, a major drawback of cassava-based by-products is the presence of hydrogen cyanide (HCN), an anti-nutritional compound known to inhibit enzymatic function, causing neurological disturbances and toxicity in livestock (Soto-Blanco and Górniak, 2010). HCN toxicity may compromise digestive and organ function animals, but these effects can be reduced through fermentation, which has been reported to decrease anti-nutritional factors and enhance nutritional value (Sugiharto and Ranjitkar, 2019; Georganas *et al*., 2023). Fermentation plays a crucial role in reducing cyanide content in cassava through multiple mechanisms. Jayanegara *et al*. (2025) reported that fermentation process creates an acidic environment, inhibiting the enzyme responsible for HCN production. Microorganisms involved during fermentation process can breakdown and detoxify cyanide, while the volatility of hydrogen cyanide allows its removal through evaporation (Brüger *et al*., 2020; Qin *et al*., 2021; Egbune at al., 2023). Lastly, the fermentation induce structural alteration in cassava cells may influence the availability of cyanogenic compounds (Montagnac *et al*., 2009). Recent studies have demonstrated that fermenting cassava by-product with *A. niger* can increase protein levels and decrease fiber content, thereby improving their potential as a livestock feed (Khempaka *et al*., 2014; Sugiharto *et al*., 2018). During fermentation, the fungus *A. niger* produces enzymes such as α-amylase, glucoamylase, and xylanase which may contribute by increasing protein content and modifying physicochemical properties of the fermented product (Aliyah *et al*., 2017).

A. niger, a filamentous fungus recognized for its enzyme-producing capabilities, which enhance the nutritional value of cassava cobs. *A. niger* produces a broad range of extracellular enzymes such as amylase, cellulase, pectinase, protease, and phytase (McKelvey and Murphy, 2017), which can breakdown complex carbohydrates and proteins, thereby improving feed digestibility (Supe, 2020). Previous studies also confirmed that *A. niger* hydrolyse tannins (Oso *et al*., 2015). Furthermore, *A. niger* synthesizes organic acids like citric acid, gallic acid, itaconic acid, oxalic acid, and gluconic acid, which contribute into breaking down cell walls and alter the chemical environment to enhance fermentation efficiency (Li *et al*., 2013; Karaffa and Kubicek, 2019; Hossain *et al*., 2019). On the other hand, citric acid not only inhibits pathogenic microbes, improve feed palatability, and modulate acidity to favor beneficial microorganism (Seo *et al*. 2013; Broom, 2015) but also lowers pH value by releasing hydrogen ion (H^+) that help improve the gut environment for the beneficial bacteria (Chuang *et al*., 2007; Latif *et al*., 2025). Moreover, *A. niger* also produce fructooligosaccharides (FOS), which exhibit significant prebiotic effect by promoting the growth of beneficial gut microbiota, thereby contributing to improved intestinal health and digestion in animals (Roupar *et al*., 2022; Mahalak *et al*., 2023). The synergistic effect of citric acid and fructooligosaccharides has been shown to significantly improve microbial and physiological conditions within the digestive tract, offering a promising approach to improve nutritional feed (Zhang *et al*., 2022; Xu *et al*., 2025).

The cassava cobs are rich in carbohydrates which serve as an excellent substrate for *A. niger* that growth optimally at 30–37°C. The fungus can effectively ferment the substrate for up to six days if sugar is available (Chew and Than, 2021; Li *et al*., 2022). However, the fermentations success depends on inoculum level and duration of fermentation, which influence the extent of microbial activity and nutrient transformation.

A. niger improves the nutritional quality of cassava cobs through solid-state fermentation (SSF) by producing hydrolytic enzymes including cellulases, xylanases, and lipases that break down fibers and enhance nutritional value (Oliveira *et al*., 2018). It also raises crude protein content, reduce lignocellulose, produces organic acids and metabolites that enhance feed safety and palatability by inhibiting pathogens and detoxifying toxins (Zhang *et al*., 2014; Wang *et al*., 2018; Cairns *et al*., 2021).

This study aimed to assess the effectiveness of *A. niger* level and fermentation durations on the nutritional profile of cassava cobs. The findings are intended to support the development of cassava cobs as a functional and sustainable alternative feed for livestock.

Materials and methods

Study location and design

This in vitro study was carried out at the Feed Technology Laboratory and the Animal Nutrition Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia. The methods were adapted by Sugiharto *et al*. (2018) with modifications. This study employed a randomized factorial design with two factors, fermentation time (0,2,4 d) and inoculum level *A. niger* (0%,2.5%,5%). Each treatment combination was replicated three times (total 27 experimental units)

Preparation of Aspergillus niger inoculum

The *A. niger* isolate was rejuvenated on Potato Dextrose Agar (PDA; Merck KGaA, Darmstadt, Germany) containing chloramphenicol and incubated at 38°C for 40 hours. Fungal mycelia were harvested by rinsing each plate with 10 mL of sterile distilled water. The resulting suspension of fungal was used to inoculated steamed rice (Miyako-PT. Kencana Sukses Gemilang) isolate: rice;1mL:10g and incubated at room temperature for 40 hours. Afterward, the culture was sun-dried and ground into powder to producing the fungal inoculum, which had a final concentration of 3.7×10^8 CFU/g.

Substrate preparation and fermentation process

Cassava cobs were collected from local farmers near the Tembalang Campus. The cassava cobs were washed, chopped, sun-dried, and ground to pass through a mesh sieve. The cassava cobs flour was sterilized using an autoclave at 121°C for 15 minutes (Utama *et al*., 2019), then cooled to room temperature. Each 150 g portion of dried cassava cob was mixed with 0.1% urea and 3% molasses (both dissolved in water) to achieve a final moisture content of 40%. The mixture was inoculated with *A. niger* at concentrations of 0, 2.5, and 5% (based on dry matter). Solid-state fermentation (SSF) was carried out under aerobic conditions for 0, 2, and 4 days at room temperature.

Nutritional and data analysis

After fermentation, cassava cob samples were analysed for changes in nutritional composition. The measured parameters included proximate composition (crude protein, crude fiber), digestible crude protein (DCP), neutral detergent fiber (NDF), acid detergent fiber (ADF), digestible NDF and ADF, cellulose, hemicellulose, and lignin contents. Data were analysed statistically to determine the effects of *A. niger* levels and time of fermentation. Standard procedures were followed AOAC, (2019), for proximate analysis and Van Soest *et al*., 1991) for fiber fraction.

All data were statistically analysed using SPSS version 27. A two-way analysis of variance (ANOVA) was conducted to examine the main effects and interaction effects between two independent variables: the level of *Aspergillus niger* inoculum and the duration of fermentation. When significant differences ($p < 0.05$) were observed, post hoc tests were performed to compare means among treatment groups. The results were interpreted to determine the optimal combination of fungal concentration and fermentation time for enhancing the nutritional value of cassava cobs.

Results

Solid state fermentation (SSF) using *Aspergillus niger* significantly altered the chemicals composition of cassava cobs. The most notable change occurred at 5% inoculum and 4 days incubation. Fiber content decreased 33.10±0.75 to 20.12±0.08, ADF content decrease 95.90±2.08 to 74.64±1.02, NDF decrease from 94.03±1.22 to 88.64±3.14 ($p < 0.05$). Crude protein content initially peaked at 42.09±0.03% on day 0 but decrease to 24.07±6.88 during fermentation at day 4 ($p < 0.05$). In contrast, digestibility crude protein significantly increased, reaching 51.00±0.59 at day 4 with 5% inoculum.

Discussion

This study demonstrates that solid-state fermentation (SSF) using *A. niger* improved the nutritional quality of cassava cobs, by reducing fiber fractions and increasing protein content digestibility. The decrease in crude fiber, NDF, and ADF content observed at 5% inoculum level and 4 days incubation indicates effective degradation lignocellulosic. This is likely due to the enzymatic activity of *A. niger* to produce cellulases, hemicellulases, and lignin-modifying enzymes (Oso *et al*., 2015), which facilitate the breakdown of complex structural carbohydrates, more digestible form (Pothiraj *et al*., 2006; Jasani *et al*., 2016). These structural changes are particularly important in improving feed nutrition. Beyond of that, in ruminant reducing ADF and NDF enhances microbial degradation in rumen, which incrases energy availability. The observed improvement in digestible NDF and ADF after fermentation support this, suggesting increase microbial utilization.

In addition to fiber degradation, increase in digestible crude protein content was observed further indicating nutritional benefit of fermentation. Although the total crude protein decline during fermentation process. This reduction is likely due to protease activity converting complex protein into simple compounds such as amino acid and peptides. While this may reduce the crude protein value measured by standard methods it improves the availability and utilization of nitrogen by the animal (Shi *et al*., 2016; Lacina and Agathos, 2006). The significant increase in digestible crude protein at 4 days fermentation support this interpretation

Consistent with the findings Akinfemi *et al*. (2009), this study demonstrated that fermentation improved the digestibility of NDF and digestibility of ADF, indicating enhanced microbial accessibility to previously difficult-to-digest fiber components. These improvements are crucial for ruminant nutrition, as they increase the energy availability from fibrous feed materials. Overall, *A. niger* effectively altered the chemical composition of cassava by-product through enzymatic degradation and biomass enrichment. The optimal treatment at 5% inoculum with 4 days of fermentation yielded the most favourable nutritional profile, with high digestible protein and reduced lignocellulosic components. These findings support the use of fungal solid-state fermentation as a sustainable strategy for improving the quality of agricultural by-products as animal feed.

Table 1. Effects of incubation time and *Aspergillus niger* inoculum levels on cassava cobs fermentation.

Parameters	Aras (%)	Fermentation duration (days)			Average
		0	2	4	
Fiber Content	0	33.10±0.75^{a}	33.17±0.86^{a}	32.07±0.08^{b}	32.78±0.78^{a}
	2.5	32.51±0.31^{ab}	26.37±0.53^{c}	24.08±0.43^{d}	27.65±3.79^{b}
	5	32.10±0.88^{b}	24.30±0.06^{d}	20.12±0.08^{e}	25.51±5.29^{c}
	Average	32.57±0.74^{a}	27.95±4.06^{b}	25.42±5.27^{c}	
Crude Protein	0	42.09±0.03^{a}	41.63±0.18^{a}	40.74±0.58^{c}	41.48±0.66^{a}
	2.5	39.59±0.24^{c}	38.82±0.31^{c}	37.06±0.56^{d}	38.49±1.17^{b}
	5	34.67±0.58^{e}	32.92±0.50f	24.07±6.88^{g}	30.55±6.01^{c}
	Average	38.78±3.28^{a}	37.79±3.86^{a}	33.96±8.33^{b}	
Digestible Fiber	0	31.18±0.16^{de}	31.44±0.39^{de}	31.46±0.28^{de}	31.36±0.29^{c}
	2.5	30.37±0.31^{e}	34.26±0.04^{c}	36.32±0.19^{b}	33.65±2.62^{b}
	5	31.71±1.21^{d}	36.32±1.09^{b}	41.00±0.60^{a}	36.34±4.11^{a}
	Average	31.09±0.86^{c}	34.01±2.20^{b}	36.26±4.14^{a}	
Digestible Crude Protein	0	41.24±0.22^{de}	41.44±0.36^{d}	41.45±0.31^{d}	41.37±0.33^{c}
	2.5	40.34±0.18^{e}	44.30±0.04^{c}	46.34±0.15^{b}	43.66±2.64^{b}
	5	41.74±1.22^{d}	46.54±0.78^{b}	51.00±0.59^{a}	46.43±4.08^{a}
	Average	41.10±0.87^{c}	44.09±2.25^{b}	46.26±4.15^{a}	
NDF[1]	0	94.03±1.22	93.64±5.34	93.46±1.07	93.71±2.80^{a}
	2.5	91.81±4.91	91.19±1.65	90.42±1.86	91.14±2.82^{a}
	5	91.14±2.82	84.35±1.86	85.68±2.52	86.64±3.14^{b}
	Average	91.91±3.29	89.72±5.10	89.85±3.77	
ADF[2]	0	95.90±2.08	92.42±2.60	90.61±2.32	92.98±3.09^{a}
	2.5	88.04±2.69	85.30±0.21	82.35±1.17	85.23±2.87^{b}
	5	81.46±1.70	78.96±1.17	74.64±1.02	78.35±3.20^{c}
	Average	88.47±6.54^{a}	85.56±6.00^{b}	82.53±7.05^{c}	
Digestible NDF	0	44.02±0.66	43.28±1.23	46.40±1.22	44.57±1.69^{c}
	2.5	47.10±0.58	47.85±0.92	48.69±2.75	47.88±1.63^{b}
	5	49.81±0.65	50.17±2.88	50.64±0.97	50.21±1.59^{a}
	Average	46.97±2.56	47.13±0.45	48.58±2.42	
Digestible ADF	0	33.42±0.68	35.7±0.37	37.43±1.15	35.51±35.51^{c}
	2.5	39.32±0.63	40.82±0.29	42.66±1.14	40.93±1.59^{b}
	5	45.01±1.18	46.14±1.27	48.95±0.65	46.70±1.99^{a}
	Average	39.25±5.08^{c}	40.89±4.57^{b}	43.01±5.07^{a}	
Cellulose	0	21.55±0.30^{a}	21.34±0.33^{a}	20.21±0.43^{b}	21.03±0.69^{a}
	2.5	19.70±0.46^{bc}	17.56±0.38^{d}	16.89±0.14^{d}	18.05±1.31^{b}
	5	19.28±0.61^{c}	17.15±0.49^{d}	16.07 ±0.61^{e}	17.50±1.50^{c}
	Average	20.18±1.12^{a}	18.68±2.03^{b}	17.72±1.93^{c}	
Hemicellulose	0	19.91±0.88^{a}	19.33±0.66^{ab}	19.15±0.52^{ab}	19.46±0.70^{a}
	2.5	18.51±0.40^{bc}	16.75±0.18^{de}	16.40±0.31^{e}	17.22±1.0^{b}
	5	17.69±0.15^{cd}	16.42±0.44^{e}	14.99±0.86^{f}	16.36±1.26^{c}
	Average	18.70±1.08^{a}	17.50±1.44^{b}	16.85±1.91^{c}	
Lignin	0	9.43±3.25	12.14±1.01	12.02±3.61	11.19±2.81
	2.5	8.93±2.57	11.42±3.62	8.18±2.18	9.51±2.88
	5	7.15±0.72	9.63±1.81	13.81±3.99	10.19±3.66
	Average	8.51±2.34	11.06±2.37	11.33±3.82	

1NDF: Neutral Detergent Fiber; 2ADF: Acid Detergent Fiber; Data are mean ± Standart Deviation;Different superscript letters in the same row indicate significant differences at $p < 0.05$.

Conclusion

A. niger effectively alter the chemical composition of cassava cobs through enzymatic degradation and biomass enrichment. The optimal treatment at 5% inoculum with 4 days of fermentation yield the most favorable nutritional profile, with high digestible protein and reduced lignocellulosic components. The results support the use of fungal solid-state fermentation as a sustainable strategy for improving the quality of agricultural by-products as animal feed.

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Conflict of interest

The authors have no conflict of interest to declare.

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