Research Article

In Vitro Evaluation of Various Treatments of Cocoa Pod Husk Bioconverted using Black Soldier Fly as a Ruminant Feed

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Abstract | The aim of this study was to evaluate the nutrient quality of cocoa pod husk (CPH) processed materials as ruminant feed. Cocoa pod husk was subjected to 4 different treatments namely T1 (control or untreated CPH); T2 (hand chopped CPH); T3 (machine chopped CPH); and T4 (fermented CPH with 3% molasses (w/w) + *Pholiota* sp. inoculum). A Completely randomized design (CRD) with four treatments and five replications per treatment was used in this study. *In vitro* cumulative gas production, *in vitro* digestibility and rumen fermentation products were observed. The collected data were analyzed using analysis of variance (ANOVA) to determine the significance of differences among treatments. The results indicated that there was no significant improvement in total *in vitro* gas production across treatments ($P > 0.05$). However, the hand-chopped treatment resulted in significantly higher optimum gas production compared to fresh cocoa pod husk (CPH) and other treatments ($P < 0.05$). All treatments applied to CPH enhanced the substrate degradation rate, which ranged from 0.079 to 0.086 ($P < 0.05$). Treatment T4 significantly improved *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of CPH ($P < 0.05$). Nevertheless, physical and biological treatments did not significantly affect pH, short-chain fatty acid (SCFA) proportions, or the metabolizable energy (ME) of CPH. It can be concluded that the nutritional quality of cocoa pod husk can be improved through fermentation using *Pholiota* sp.

Keywords | Black soldier fly, Chopped, Cocoa pod husk, *In vitro* digestibility, *Pholiota* sp.

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INTRODUCTION

Indonesia ranks as the sixth largest cocoa bean producer globally in the 2018/2019 period, following Ivory Coast, Ghana, Ecuador, Cameroon, and Nigeria (Shahbandeh, 2021). Cocoa pod is the residue product of cocoa plants with the proportion reaching 75% of fresh fruit (Laconi and Jayanegara, 2015; Yakin *et al*., 2021). After the cocoa bean is separated from the fruit, cocoa pod husk (CPH) is produced as the main residue (Syamsiro *et al*., 2012). Despite its abundance, CPH remains underutilized by farmers and is often left to decompose, potentially becoming a breeding ground for *Phytophthora palmivora*, the pathogen responsible for black pod disease. Effective utilization of CPH, particularly as livestock feed, could not only mitigate environmental issues but also enhance the economic value of cocoa plantations (Nurhaita *et al*., 2018). One of the primary limitations of CPH as a ruminant feed is its high

lignin content (31.51%), which hinders digestibility and nutrient absorption in the rumen (Nurhaita *et al*., 2015). Additionally, CPH is characterized by high levels of neutral detergent fiber (NDF; 55–73.90%) and acid detergent fiber (ADF; 38.31–59.98%) (Puastuti and Susana, 2014). Such composition reflects the common characteristics of agricultural by-products: fibrous and protein-deficient (Laconi and Jayanegara, 2015). Thus, processing methods are essential to improve its nutritional quality.

Various strategies have been proposed to process CPH, including physical, chemical, and biological treatments (Puastuti and Susana, 2014). Combining fiber-cracking technologies with urea treatment has been shown to enhance the quality of agricultural residues (Dewi *et al*., 2018). Environmentally friendly approaches, such as biological and physical treatments, are particularly promising. Fermentation using fungi such as *Aspergillus oryzae*, *A. niger*, *Phanerochaete chrysosporium*, *P. ostreatus* and *Trametes versicolor* can degrade lignin and improve protein content while reducing crude fiber, NDF, and ADF levels (Syahrir *et al*., 2013; Rakhmani and Purwadaria, 2017; Yakin *et al*., 2021). Supplementing fermented CPH with minerals (S, P, Zn) has also been shown to improve *in vitro* digestibility (Nurhaita *et al*., 2018). Moreover, previous studies have demonstrated that rations incorporating fermented CPH significantly improve dry matter intake, nutrient digestibility and growth performance in ruminants compared to unprocessed CPH (Laconi and Jayanegara, 2015; Yakin *et al*., 2021).

While several fungi have been explored for fermentation, *Pholiota* sp. remains underutilized despite its demonstrated potential in degrading lignocellulosic biomass (Musatti *et al*., 2017) and reducing anti-nutritional factors such as phytate (Jatuwong *et al*., 2020). Comparative studies evaluating *Pholiota* sp. alongside physical treatments like chopping remain limited and thus warrant further investigation to optimize CPH utilization. Another emerging biotechnology for agricultural waste valorization is the use of *Black Soldier Fly* (BSF; *Hermetia illucens L.*) larvae for bioconversion. BSF larvae efficiently convert organic materials—including food waste (Spranghers *et al*., 2017), livestock manure (Cockcroft, 2018; Rehman *et al*., 2017) and various agricultural by-products (Manurung *et al*., 2016; Supriyatna *et al*., 2016)—into high-value biomass. This biomass is rich in protein, with levels reaching up to 40%, making BSF an attractive agent for sustainable feed production (Elwert *et al*., 2010; Finke, 2013; Jayanegara *et al*., 2017). Given these potentials, this study aims to evaluate the nutrient quality of CPH processed through various biological and physical treatments, including bioconversion using BSF larvae. *In vitro* cumulative gas production, digestibility and rumen fermentation profiles were assessed as indicators of nutritional improvement.

MATERIALS AND METHODS

Study Site and Research Timeline

This study was conducted at the Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, located in Bogor, Indonesia (Latitude: -6.5897, Longitude: 106.8019). The experimental site features a tropical climate with an average temperature of 27–30°C and humidity levels of 80–90%. The soil in the area is classified as andosol, characterized by high porosity and fertile volcanic ash content, suitable for diverse agricultural activities.

The experimental procedures including sample preparation, fermentation, BSF bioconversion, and in vitro evaluations were carried out between July and November 2023. Laboratory analyses were performed at the Animal Feed Chemistry and Ruminant Nutrition Laboratory. The ambient room temperature during processing and incubation averaged 27–30°C with 80–90% relative humidity.

Raw Materials

Cocoa pod was obtained from cocoa farm, Nusantara Plantation VIII-Public Company (PTPN VIII), Indonesia. One kg of cocoa pod was used for each treatment. The fresh pods were collected from mature cocoa plants, transported on the same day, and stored at ambient temperature prior to processing. Fresh CPH was chopped manually around 1 cm thickness and subjected to T1 treatment. Pholiota sp. inoculum was obtained from Indonesian Research Institute for Biotechnology and Bioindustry, Bogor, Indonesia. Molasses was purchased from a commercial market.

Experimental Treatments

This study employed a completely randomized design (CRD) with four treatments, each replicated five times. The study design consist of:

- T1: Fresh Cocoa Pod Husk (CPH)
- T2: Hand chopped CPH
- T3: Machine chopped CPH
- T4: Fermented CPH with 3% molasses (w/w) + *Pholiota* sp. inoculum

For T2 treatment, fresh CPH was chopped manually around > 2 mm thickness. For T3 treatment, fresh CPH samples of 200 g was blended for one minute with distilled water using a kitchen blender (Cosmos CB-287, Cosmos). In the case of T4 treatment, 300 g of CPH was placed in polyethylene bags and kept aerobically at room temperature for 21 d. All samples were subsequently subjected to a 14-day bioconversion process using Black Soldier Fly (BSF) larvae (Hermetia illucens L.), reared until the pupal stage. The rearing process was conducted in ventilated

plastic containers at room temperature, with daily observation to ensure optimal larval activity.

Prior to *in vitro* evaluation, all treatments were used as BSF media until the pupa stage was carried out for 14 days and then continued with *in vitro* evaluation. Representative samples (0.75 g) were dried at 65°C for 48 h and analyzed for proximate composition, i.e. ash, organic matter (OM), crude protein (CP), ether extract (EE) and crude fiber (CF) by following the procedure from Association of Official Analytical Chemists method (AOAC, 2005). The initial proximate compositions of the processed CPH materials are summarized in Table 1.

Table 1: The nutrient contents of fresh and treated cocoa pod husk (CPH).

	% Dry matter				
Treatment	Organic matter	Ash	Crude protein	Ether extract	Crude fiber
T1	82.46	17.54	16.21	1.12	27.71
T2	82.33	17.76	16.45	0.67	35.26
T3	80.69	19.31	15.69	0.71	40.18
T4	75.27	24.73	15.47	0.72	37.24

Note: T1: fresh cocoa pod husk (CPH); **T2:** hand chopped CPH; **T3:** machine chopped CPH; **T4:** fermented CPH.

In-vitro Evaluation

A 0.75 g sample was incubated with a buffered rumen fluid mixture following the procedure described by Theodorou *et al.* (1994). Rumen fluid was collected before morning feeding from a rumen-fistulated goat maintained at the Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University. To guarantee uniformity, the same volume of buffer and rumen fluid mixture was used for each incubation, and all samples were incubated under identical conditions**.** Before use, rumen fluid was filtered through four layers of cheesecloth. An amount of 750 mg sample was inserted into a 125 ml serum bottle and added with 75 ml buffered rumen fluid (rumen fluid to buffer ratio was 1:4 v/v). Serum bottles were the sealed with butyl rubber stoppers and aluminum crimp seals to start the incubation. Incubation was performed in a water bath maintained at 39 °C for 72 h. Gas production was recorded at 2, 4, 6, 8, 12, 24, 36, 48 and 72 h. Manual shaking was conducted at each time of gas production recording. Exponential Ørskov equation (Ørskov and Mcdonald, 1979) was used to observe kinetics gas characteristics, as follows: $P = a + b(1 - e^{-ct})$, where P represents gas production at t time, a is cumulative gas production from soluble fraction, b is cumulative gas production from non-soluble fraction and c is the rate of gas production.

In vitro dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were calculated by subtracting DM and OM residues from their initial values prior to incubation. Three independent incubation runs were conducted, and each treatment was represented by two bottles per run. Allocation of treatments into experimental units followed a randomized complete block design in which different *in vitro* runs served as the blocks (replicates). Two bottles per run without any substrate but containing buffered rumen fluid were also incubated to serve as blanks.

An amount of 20 ml of *in vitro* medium was collected after 72 hours of incubation to determine pH. The pH was measured using a digital pH meter (Hanna Instruments, HI 2211). Metabolizable energy (ME) after 24 h incubation were determined according to (Menke *et al.*, 1979) calculations, as follows:

$$ME\ (MJ/kg\ DM) = 2.20 + (0.136 \times gas\ production\ at\ 24\ h) + (0.057 \times CP)$$

By maintaining equal sample sizes and standardized procedures across all treatments, this methodology aims to ensure a fair and accurate comparison of the effects of each treatment on CPH, with each parameter analyzed using four replicates per treatment to enhance statistical reliability and minimize variability.

Statistical Analysis

Experimental data were analyzed using analysis of variance (ANOVA), preceded by a normality test to ensure that the data met the assumptions required for parametric analysis. Duncan's multiple range test was used to determined means comparison between treatments. Significant levels were accepted at $P<0.05$. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

This study evaluated the effects of physical and biological treatments on the nutritional quality, gas production kinetics, digestibility and rumen fermentation characteristics of cocoa pod husk (CPH) bioconverted using Black Soldier Fly (BSF) larvae. The treatments included: T1 (fresh/unprocessed CPH), T2 (hand-chopped CPH), T3 (machine-chopped CPH) and T4 (fermented CPH with 3% molasses and Pholiota sp. inoculum). The results are presented through proximate analysis (Table 1), gas production kinetics (Table 2), digestibility and fermentation characteristics (Table 3), and cumulative gas production over time (Figure 1).

Table 1 presents the proximate composition of the CPH before *in vitro* incubation. The treatments had varying effects on dry matter (DM), ash, organic matter (OM), crude

Table 2: Differences in the *in vitro* gas characteristics of fresh and treated cocoa pod husk (CPH).

Treatment	Cumulative gas production (ml/750 mg DM)								
	Incubation time (h)								
	2	4	6	8	10	12	24	48	72
T1	9.51^{a}	16.59^{ab}	22.03^{c}	23.28^{b}	24.07^{c}	24.64^{c}	25.88^{b}	28.77	30.76^{ab}
T2	12.78^{c}	18.05^{b}	20.29^{bc}	21.02^{a}	22.14^{bc}	22.87^{bc}	26.46^{b}	29.14	32.45^{b}
T3	9.23^{a}	15.16^{a}	18.01^{a}	18.96^{a}	19.52^{a}	20.08^{a}	23.66^{a}	27.85	30.31^{a}
T4	11.31^{b}	18.19^{b}	18.74^{ab}	19.30^{a}	20.85^{ab}	21.40^{ab}	23.51^{a}	27.06	29.39^{a}
SEM	0.333	0.362	0.431	0.436	0.446	0.450	0.417	0.356	0.384

Note: T1: fresh cocoa pod husk (CPH); **T2:** hand chopped CPH; **T3:** machine chopped CPH; **T4:** fermented CPH. Means with the different letters in the same column are significantly different (P<0.05). **DM:** Dry matter.

Table 3: Differences in the *in vitro* digestibility and rumen fermentation product of fresh and treated cocoa pod husk (CPH).

Treatment	Optimum Gas Production (a+b)	Rate of Gas Production (c)	IVDMD	IVOMD	SCFA (mM)			ME	pH
	(ml/750 mg DM)		(%)	(%)	C_2	C_3	C_4	(MJ/kg DM)	
T1	28.32^{a}	0.212^{b}	41.13^{a}	28.94^{a}	20.26	5.89	3.82	6.64^{b}	7.18
T2	31.97^{b}	0.079^{a}	40.74^{a}	28.39^{a}	21.84	7.19	4.71	6.74^{b}	7.19
T3	29.12^{a}	0.079^{a}	43.57^{b}	38.55^{b}	22.12	6.42	3.98	6.29^{a}	7.20
T4	28.27^{a}	0.086^{a}	48.31^{c}	50.32^{c}	23.92	6.31	4.16	6.29^{a}	7.17
SEM	0.461	0.011	0.526	1.466	0.800	0.232	0.156	0.068	0.038

Note: T1: fresh cocoa pod husk (CPH); **T2:** hand chopped CPH; **T3:** machine chopped CPH; **T4:** fermented CPH. **IVDMD:** *in vitro* dry matter digestibility; **IVOMD:** *in vitro* organic matter digestibility; **SCFA:** short chain fatty acids; **C_2:** acetate; **C_3:** propionate; **C_4:** butyrate; **ME:** metabolisable energy. Means with the different letters in the same column are significantly different (P<0.05).

protein (CP), ether extract (EE), and crude fiber (CF). The dry matter content ranged from 89.33% (T3) to 91.04% (T2), with no significant difference (P>0.05) among treatments. Ash content was consistent across treatments, ranging from 11.73% (T2) to 12.56% (T4) (P>0.05). Organic matter values also showed no significant differences (P>0.05), with values ranging from 87.44% (T4) to 88.27% (T2). Crude protein content ranged from 7.28% (T1) to 9.87% (T4), with T4 showing significantly higher CP than T1 and T2 (P<0.05). Ether extract values varied between 2.14% (T4) and 2.78% (T3), but the differences were not statistically significant (P>0.05). Crude fiber content was highest in T1 (30.81%) and lowest in T3 (28.03%), with no significant reductions observed across treatments (P>0.05). Overall, while fermentation and chopping affected CP content, CF levels remained statistically unchanged.

Cumulative gas production during 72-hour incubation is presented in Figure 1. All treatments exhibited a rapid increase in gas volume during the first 8 hours of incubation, followed by a gradual decline in gas production rate. The highest gas production was generally recorded within 8 to 12 hours. At 2, 4, 6, 8, 12, 24, and 36 hours, the cumulative gas production showed significant differences (P<0.05) among treatments. At 48 hours, no significant difference was found (P>0.05). By the end of the 72-hour incubation, cumulative gas production was significantly higher (P<0.05) in T2 (22.3 mL) than in T1 (17.5 mL), T3 (20.1 mL), and T4 (19.8 mL). This indicates that hand-chopping improved early fermentation activity. However, fermentation with Pholiota sp. (T4) did not significantly increase cumulative gas production at 72 h compared to untreated or chopped treatments (P>0.05).

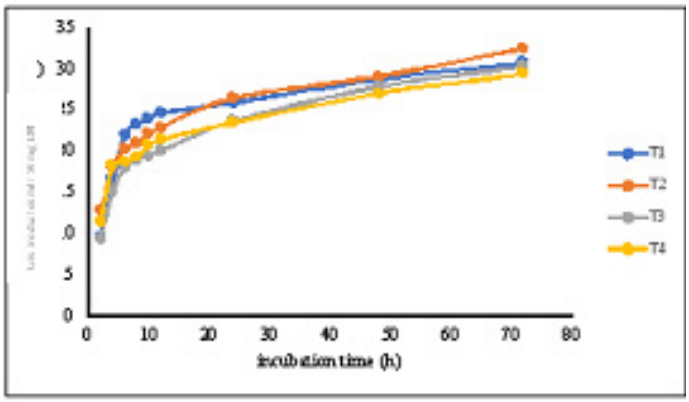

Figure 1: *In vitro* gas production of fresh and treated cocoa pod husk (CPH). **T1:** fresh cocoa pod husk (CPH); **T2:** hand chopped CPH; **T3:** machine chopped CPH; **T4:** fermented CPH; **DM:** Dry matter.

Table 2 summarizes the gas production kinetics parameters estimated using the Ørskov and McDonald model, including the immediately soluble fraction (a), the insoluble fraction (b), the potential gas production (a + b), and the rate constant (c). The soluble fraction (a) did not differ significantly among treatments (P>0.05), ranging from 2.13 mL (T1) to 2.46 mL (T4). The insoluble fraction (b) was highest in T2 (19.81 mL) and significantly greater than that of T1 (15.45 mL) (P<0.05). The potential gas production (a + b) showed a significant difference (P<0.05), with T2 (22.27 mL) being significantly higher than T1 (17.58 mL). T3 and T4 had intermediate values and were not significantly different from T1 or T2 (P>0.05). The degradation rate constant (c) varied significantly among treatments (P<0.05). All processed CPH (T2, T3, and T4) had significantly higher values (0.079–0.086 h^{-1}) compared to T1 (0.068 h^{-1}), indicating faster substrate degradation due to processing.

Table 3 presents data on *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), pH, and short-chain fatty acids (SCFA). IVDMD values ranged from 34.18% (T1) to 48.17% (T4). T4 had the highest IVDMD and IVOMD values, significantly higher than all other treatments (P<0.05). IVOMD ranged from 29.41% (T1) to 42.67% (T4), following the same trend. T2 and T3 also showed significantly higher digestibility than T1 (P<0.05), though lower than T4. Metabolizable Energy (ME) values ranged from 6.33 MJ/kg DM (T4) to 8.01 MJ/kg DM (T2). T1 and T2 had significantly higher ME values than T3 and T4 (P<0.05), despite their lower digestibility. T4, despite its higher digestibility, showed the lowest ME value. The final pH values after 72 hours of incubation were similar across all treatments, ranging from 7.17 to 7.18 (P>0.05). No treatment caused significant alterations in ruminal pH, and all values remained within the neutral range. The SCFA concentrations were significantly different across treatments (P<0.05). T2 had the highest SCFA (19.32 mM), followed by T1 (17.83 mM), T3 (15.24 mM) and T4 (13.43 mM). SCFA values in T3 and T4 were significantly lower than in T1 and T2 (P<0.05).

The current study offers valuable insights into the effects of various physical and biological treatments on cocoa pod husk (CPH)—a significant agricultural by-product—aiming to enhance its digestibility and nutritional value as a ruminant feed. The multifaceted approach, incorporating chopping, fermentation with fungi (notably *Pholiota* sp.), and bioconversion via Black Soldier Fly *(Hermetia illucens)*, provides a comprehensive perspective on valorizing this underutilized resource. The results reveal nuanced effects on fermentation kinetics, digestibility parameters, and gas production profiles, which merit a thorough and critical discussion grounded in prior literature, underlining both the potentials and constraints associated with these processing strategies.

Physical disruption, particularly chopping, emerged as a prominent intervention, significantly increasing the substrate's fermentability, as evidenced by the higher optimum gas production (a+b) compared to untreated CPH. This aligns with previous findings by Gallo *et al*. (2018), who demonstrated a linear relationship between decreasing particle size and enhanced fermentation rate, attributable to increased surface area facilitating microbial attachment and enzymatic activity. Particle size reduction is also known to facilitate initial hydrolysis of complex fiber matrices, thus improving the accessibility of cellulolytic bacteria and enzymes to polysaccharides, which is instrumental for fermentative degradation.

However, while chopping improves fermentation kinetics, its impact on overall fiber digestibility remains limited unless combined with other treatments. The inability of chopping alone to significantly reduce lignin content—a critical barrier to fiber digestibility—is a well-known limitation, as lignin's physical and chemical recalcitrance in fibrous materials remains largely unaffected by mechanical means (Menke *et al*., 1979). The implication is that physical treatment alone may primarily accelerate initial fermentation phases but insufficiently promote complete fiber breakdown, especially when lignocellulosic bonds are intact.

Nevertheless, the data corroborate that physical processing is an essential first step, potentially enhancing the efficacy of subsequent biological or chemical treatments. Integrating physical disruption with biological agents—such as fungi capable of lignin modification—may synergetically augment digestibility, as previous research has suggested (Dewi *et al*., 2018). From an operational standpoint, particle size control remains a practical, low-cost, and scalable approach to improve fermentability, especially in resource-limited settings.

Fungal fermentation, particularly with *Pholiota* sp., showed promising improvements in *in vitro* digestibility parameters (IVDMD and IVOMD) despite not significantly increasing total gas production. This observation implies that fungal enzymes modify the fiber structure, making carbohydrates more accessible without proportionally increasing fermentation volume. The ligninolytic capacity of *Pholiota* sp., a basidiomycete, produces lignin-degrading enzymes such as laccases, exoglucanases, xylanases, and endoglucanases (Musatti *et al*., 2017). Such enzymatic activity enhances the breakdown of lignin-cellulose bonds, leading to improved nutrient availability—consistent with the find-

ings of Jutawong *et al.* (2020), who reported that phytase and cellulolytic enzyme activity effectively enhance nutrient digestibility.

However, the extent of lignin degradation in this study appears limited, possibly due to the specific enzyme profile of *Pholiota* sp., incubation duration, or substrate recalcitrance. Since lignin is hydrophobic and chemically resistant, complete removal or significant alteration often requires extended incubation periods or co-treatment with chemical agents (Musatti *et al.*, 2017). Furthermore, the effectiveness of fungal treatments is highly strain-dependent; other fungi, such as *Phanerochaete chrysosporium* have demonstrated superior ligninolytic activity, suggesting opportunities for future research to optimize enzyme production and treatment duration.

An important implication of fungal treatment is the potential reduction of antinutritional factors such as phytates, which sequester essential minerals, thus improving mineral bioavailability (Nurhaita *et al.*, 2018; Jatuwong *et al.*, 2020). The enzymatic breakdown of phytates and fiber bonds not only enhances digestibility but potentially supports better growth performance in ruminants, aligning with prior research indicating~that fermented agricultural residues outperform unprocessed feeds (Laconi and Jayanegara, 2015; Zhong *et al.*, 2016). Nonetheless, the durability and scalability of fungal fermentation require further validation, including *in vivo* trials and cost-benefit analyses.

Total gas volume, a common indicator of fermentation extent, was not significantly affected by fungal fermentation but was notably higher in chopped treatments, reaffirming the physical effect of particle size reduction. The rate of degradation (c) increased consistently across treatments, indicating a faster initial hydrolysis phase. This acceleration can be linked to increased enzyme-substrate contact surface, corroborating the observations by Gallo *et al.* (2016; 2018). Increased degradation rates are particularly advantageous in practical feeding scenarios, where faster fermentation can result in more efficient feed utilization and reduced feed passage time.

The absence of a significant increase in total gas volume in fermented treatments, despite improved digestibility, suggests that biotic modifications may shift fermentation pathways towards more efficient carbohydrate utilization, without necessarily increasing total gas output. This is critical because total gas production, especially methane, poses environmental concerns, although methane measurements were not directly conducted in this study. Future studies should incorporate methane quantification, as highlighted by Wahyono *et al.* (2019; 2020), to evaluate the environmental sustainability of processing methods.

The observed changes, including decreased VFAs in fermented samples, imply altered fermentation pathways or microbial profiles, possibly favoring fibrolytic bacteria and fungi that may produce different metabolite profiles. Since VFA profiles influence energy supply—and hence animal performance—further metabolomic analysis could provide deeper insights into how these treatments modify fermentation end-products *in vivo* (McDonald *et al.*, 2010).

Despite microbial enzyme activity, the crude fiber (CF) content remained relatively stable across treatments. These findings agree with previous reports (Musatti *et al.*, 2017) that enzymes produced by fungi like *Pholiota* sp. primarily target hemicellulose and lignin-linked bonds rather than drastically reducing lignin content. Lignin's complex aromatic structure renders it highly resistant, and its removal often requires aggressive pretreatments, such as alkaline or oxidative chemical methods, which may not be feasible or sustainable at scale.

The limited reduction in CF and lignin suggests that fungal fermentation needs to be coupled with other strategies, such as chemical delignification or biological consortia combining lignin-degrading fungi with cellulolytic bacteria, to realize more substantial improvements. Furthermore, the fiber composition data indicate that more specialized fungal strains or longer incubation times could be explored to enhance lignin degradation further, enabling better access to digestible polysaccharides.

The critical challenge in utilizing CPH as ruminant feed lies in its high lignin, NDF, and ADF content—attributes that diminish digestibility. The present findings suggest that processing strategies—especially physical chopping and fungal fermentation—can partially ameliorate these issues. Specifically, fungal treatments appear to modify fiber bonds, boosting digestibility, which aligns with the work of Syahrir *et al.* (2013) who documented increased digestibility with fermentation using Pleurotus spp., and with Laconi and Jayanegara (2015), emphasizing fermentation's role in amplifying nutrient availability.

Furthermore, these treatments might also influence animal health and productivity by reducing the intake of indigestible fibers, decreasing feed passage time, and improving nutrient absorption. However, the static nature of CF and lignin contents indicates room for improvement, perhaps through combined physical, biological, and chemical pretreatments, to push the boundaries of digestibility.

From an environmental perspective, the potential reduction in methane emissions—although not directly measured—could be significant. As gas production is closely linked to methanogenesis pathways, strategies that en-

hance fermentation efficiency and modulate VFA profiles can potentially mitigate greenhouse gas emissions. Future research integrating *in vivo* methane measurement, alongside digestibility and performance metrics, will be crucial to establishing environmental benefits.

While the study provides meaningful insights, it inherently bears limitations. The *in vitro* nature restricts extrapolation to *in vivo* systems where animal factors—such as saliva flow, passage rate, and microbiota diversity—play pivotal roles. Moreover, the incubation duration and fungal inoculum conditions may not fully represent optimal parameters for lignin degradation or nutrient solubilization. The fungal strain used, *Pholiota* sp., offers a promising starting point, but exploring other ligninolytic fungi with higher enzymatic activity—like *Phanerochaete chrysosporium*—could enhance fiber breakdown substantially (Musatti *et al*., 2017). The possible synergies between physical mechanical treatments and biologically active fungi should be investigated further, perhaps through multi-step treatments combining mechanical size reduction, fungal inoculation, and chemical delignification to maximize digestibility gains.

Additionally, comprehensive chemical analyses—such as lignin composition, phenolic contents, and enzyme activity profiling—would deepen understanding of the mechanisms underpinning digestibility improvements. Careful *in vivo* trials are imperative to evaluate feed intake, growth performance, health parameters, and methane emissions, translating *in vitro* results into

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates that fermenting cocoa pod husk (CPH) with *Pholiota* sp. enhances its digestibility and nutritional quality, making it a valuable alternative feed resource for livestock in cocoa-producing regions. Livestock farmers, feed producers, and policymakers can incorporate fermented CPH into ruminant diets at recommended levels—potentially up to 20-30% of the total feed—thereby improving nutrient absorption and animal performance. The higher digestibility associated with fermentation is expected to increase feed efficiency, promote growth, and boost productivity in terms of weight gain and milk or meat output, ultimately benefiting farm profitability. To optimize utilization, further *in vivo* trials and economic assessments are recommended, along with extension services to train farmers in fermentation techniques. Overall, adopting fermented CPH as a feed ingredient supports sustainable livestock practices, reduces feed costs, and contributes to waste valorization, food security, and rural economic development.

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NOVELITY STATEMENT

This study introduces a novel approach to improving the nutritional quality of cocoa pod husk (CPH), an underutilized agricultural by-product, through the integration of physical (chopping) and biological (fermentation with Pholiota sp. and bioconversion using Black Soldier Fly larvae) treatments. While previous research has explored the benefits of individual treatment methods, this work uniquely combines these strategies to assess their collective impact on in vitro digestibility, gas production kinetics, and rumen fermentation characteristics. The findings demonstrate that combining these methods significantly enhances the digestibility of CPH, offering a promising alternative feed resource for ruminants. Moreover, this study is one of the first to evaluate the potential of Pholiota sp. fermentation in improving CPH as ruminant feed, filling a gap in the literature and providing a sustainable solution to the challenges posed by high lignin content in agricultural residues.

AUTHOR'S CONTRIBUTIONS

Rahman Rahman: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization.
Erika Budiarti Laconi: Supervision, Methodology, Validation, Writing – review and editing, Resources.
Dewi Apri Astuti: Supervision, Project administration, Writing – review and editing, Funding acquisition.
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All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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