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BIOCONVERSION OF CEREAL BY-PRODUCTS THROUGH *Pleurotus ostreatus* MEDIATED SOLID-STATE FERMENTATION OF THREE AGRO BY-PRODUCTS

Dennis AFRAM[1]✉, Emmanuella Ekua Tweneboah GYAMFI[1], Jennifer VAN-ESS[1], Nene WILLIAMS[1], Victoria ATTOH-KOTOKU[1], Agyemang Felix ABOAGYE[1], Kenneth Gyimah OWUSU[1], Prince SASU[1], Benjamin ADJEI-MENSAH[2], and Armstrong DONKOH[1]

[1]*Department of Animal Science, Faculty of Agriculture, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2]*Department of Animal Science, University of Ghana, Legon, Accra, Ghana*

✉Email: aframdennis1@gmail.com

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ABSTRACT: Solid-state fermentation (SSF) offers a promising approach to enhance the nutritional value of abundant but underutilized agro-industrial by-products in sub-Saharan Africa. The study investigated the effect of SSF using *Pleurotus ostreatus* on rice bran (RB), rice husk (RH) and maize cob (MC). The design used was a 3 × 3 factorial arrangement of treatments in a completely randomized design (CRD). The factors were three substrates (RB: rice bran, RH: rice husk, and MC: maize cob) and three fermentation periods (0, 30, 45 days). Unfermented and fermented samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). *In vitro* gas production (3-96 h) was measured using rumen liquor. *In vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), net energy (NE), short-chain fatty acids (SCFAs), and microbial protein (MP) were estimated using established models. Significant differences ($P < 0.05$) in CP and EE contents were observed among RB, RH, and MC within fermentation periods. The CP contents at days 30 and 45 were 9.44%, 8.10% for RB, 3.07%, 4.69% for RH, and 5.32%, 12.19% for MC, respectively. Fibre fractions varied among substrates at each fermentation period, with NDF differing among RB, RH, and MC, and ADL showing significant differences among substrates at day 30. Potential gas production (*b*) rose in MC (52.7 ml) and RH (53.9 ml) after 45 days. The rate of gas production (c) ranged from 0.03-0.08 h^{-1} at day 0 and 30, but after 45 days, RH reached 0.08 h^{-1}, while RB and MC slowed to 0.01 h^{-1}. IVOMD, ME, NE, SCFA and MP were all higher in fermented RH and MC, with peak improvements at day 30. The study results indicated that SSF for 30-45 days could improve the nutritional value of agro-industrial by-products.

Keywords: Chemical composition, *In vitro* gas production, Maize cob, Rice bran, Rice husk, Oyster mushroom, Solid-state fermentation.

INTRODUCTION

The rise in global demand for sustainable livestock production has made researchers focus on developing nutritional feed resources from agricultural by-products. Multiple agricultural by-products, including rice bran, rice husk and maize cob, are abundant feed resources, but their use in ruminant diets is limited by low nutritional value and high lignocellulosic content (Omarini et al., 2019; Fidriyanto et al., 2020). The high fibre and lignin content of these by-products limits nutrient absorption and reduces digestibility, posing a challenge to ruminant production, particularly in regions where feed is scarce and conventional ingredients are expensive (Sufyan et al., 2021).

Rice bran, due to its fat and protein content, is prone to rancidity, whereas rice husk and maize cob are rich in cellulose, hemicellulose and lignin, resulting in low protein and energy availability (Omarini et al., 2019; Fidriyanto et al., 2020). The high crude protein content of rice bran makes it optimum for use, whereas the fibrous nature reduces the overall feed value of these by-products (Wang et al., 2012; Liu et al., 2023). In developing countries, limited resources constrain ruminant production, creating a need for cost-effective technologies to convert agricultural residues into high-quality animal feeds (Wang et al., 2023). To address this need, enhancing the nutritional value of agro-industrial wastes through microbial bioconversion has emerged as a promising approach, particularly via the biotechnological process of solid-state fermentation (SSF).

White-rot fungi, specifically *Pleurotus* species, exhibit high potential in biological transformations due to their filamentous growth (Eliopoulos et al., 2022). Their lignocellulolytic enzymes selectively degrade lignin, thereby releasing cellulose and hemicellulose for improved digestibility (Eliopoulos et al., 2022). Additionally, these fungi reduce anti-nutritional factors in raw substrates while contributing enzymes, mycelial biomass and bioactive compounds that enhance rumen fermentation (Wang et al., 2023).

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Previous research has demonstrated that solid-state fermentation (SSF) using *Pleurotus* species can significantly enhance the nutritional value of agricultural by-products. Fermentation of rice bran with *Pleurotus sapidus* significantly increased protein and reduced fiber fractions, enhancing ruminal digestibility (Omarini et al., 2019). Sufyan et al. (2021) demonstrated that applying solid-state fermentation (SSF) to cereal straws and corn by-products significantly enhanced crude protein concentration and improved *in vitro* digestibility characteristics. Solid-state fermentation (SSF) using *Pleurotus* species has been shown to degrade lignin and enhance protein content, improving digestibility and energy availability of agro-industrial residues (Eliopoulos et al., 2022). Although SSF has been applied to various cereal and corn by-products, limited information exists regarding its effects on rice bran, rice husk, and maize cob, particularly using *Pleurotus ostreatus*. Therefore, this study aims to evaluate the effect of SSF using *Pleurotus ostreatus* mycelia on the chemical composition and in vitro fermentation characteristics of these by-products.

MATERIALS AND METHODS

Location and climate of the experimental site

The study was carried out at the Nutrition and Pathology Laboratories of the Departments of Animal Science and Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The site lies in South Eastern Kumasi at 6°43′N latitude, 1°36′W longitude and an elevation of 261 m above sea level. The area experiences bimodal rainfall, with an annual mean of 1194 mm and average temperatures ranging from 22.3°C to 31.9°C.

Source of materials

The samples were obtained and randomly collected from vendors at the Zongo local market within the Ejisu community. The oyster mushroom for the solid-state fermentation was purchased from the Fresh Logistic Greenhouse, Mango Road, Kwame Nkrumah University of Science and Technology (KNUST).

Preparation of materials

Rice bran, rice husk and maize cob were air-dried under shade for two weeks to reduce moisture content and prevent nutrient loss. The rice bran, which had the 2mm particle size was packed after drying. The rice husk and maize cob after drying were milled to 2 mm sieve sizes using a laboratory Hammer Mill. Fresh oyster mushrooms (*Pleurotus ostreatus*) were surface sterilized with cotton wool moistened in 70% ethanol. The gills were gently opened to expose internal tissue, and a small piece of pileus was aseptically transferred to sterile potato dextrose agar (PDA) plates using sterile forceps. The plates were incubated at room temperature (25–28 °C) for 14 days until full mycelial growth was observed. This procedure ensured the establishment of a pure culture for subsequent solid-state fermentation experiments. Figures 1 and 2 show the experimental image used in the study.

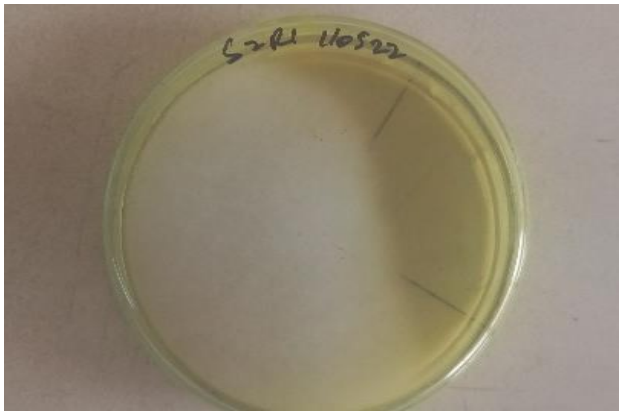

Figure 1 - Oyster mushroom culture

Figure 2 - Oyster mushroom

Experimental design

A 3×3 factorial arrangement in a completely randomised design (CRD) was used. The factors were three fermentation periods 0, 30 and 45 days and three by-products Rice husk, maize cob and rice bran. There were 9 treatments with three replications each. The treatments were Rice bran at day zero, day 30 and day 45 after fermentation; Rice husk at day zero, days 30 and 45 after fermentation and Maize cob at day zero, day 30 and day 45 after fermentation.

Chemical analyses

The samples underwent proximate analyses using the procedures of AOAC (1990) to determine dry matter (DM), crude protein (CP), ash, CF and ether extract (EE). The NDF, ADF and ADL contents were determined using the Sulfite method of Van Soest et al. (1991).

In vitro gas production

Rumen liquor was collected from three ewes (*Ovis aries*) slaughtered at the Kumasi Abattoir, Ghana. The animals were maintained on a grass diet and starved for 12 hours before slaughter. Ten minutes after slaughter and removal of the visceral organs, rumen liquor was collected into pre-warmed flasks, tightly sealed and promptly transported to the laboratory for analysis. *In vitro* gas production measurements were carried out using the method of Menke and Steingass (1988) in which 200 mg dried samples were incubated three times in a water bath at a temperature of 39°C, with 20 ml of artificial saliva, bicarbonate buffer, macro- and micro- minerals, resazurin, a reducing solution and 10 ml of rumen liquor. The volume of the gas was measured as the displacement of the syringe plunger at 0, 3, 6, 12, 24, 48, 72, and 96 h of incubation. SigmaPlot for Windows (version 15.0) software (Systat Software Inc., 2017) was used to fit each gas reading into the model $Y = b\ (1-e^{-ct})$.

Y =volume of gas produced at time t(ml); b = the potential possible gas produced from the insoluble fraction (ml/200 mg DM); c = rate at which gas was produced from insoluble fibres (ml/hr), and t = incubation time.

In vitro organic matter digestibility (%), metabolisable (ME MJ/kg DM), net energy (NE, MJ/kg DM), short-chain fatty acids (SCFA), and microbial protein (MP) were also calculated using the stoichiometric equations presented by Menke and Steingass (1988) and Close and Menke (1986):

ME (MJ/kg DM) = (0.136 IVGP24) + 0.057 X CP + 2.20, ... (1)

NE (MJ/kg DM) =[(0.149×EE) +(0.057×CP) + (0.0272×IVGP24) +2.20] ÷14.64, ... (2)

MP = IVOMD×1.93 ÷10, ... (3)

SCFA = [-0.0222xIVGP24 (ml/0.5g DM) X 100], ... (4)

IVOMD (%) = [0.889 IVGP24 +0.0651 Ash+0.45CP (%DM) +14.88], ... (5)

Note: IVGP24 is the volume of gas produced after 24 hours of incubation.

Statistical analyses

The data were analyzed using a completely randomized design and subjected to analysis of variance (ANOVA). The Statistical Package for Social Scientists (SPSS), version 20 (IBM Corp., 2011), was used, and mean separation was performed using Tukey Pairwise comparison at a 5% threshold for declaring significance. SigmaPlot for Windows (version 15.0) software (Systat Software Inc., 2017) was used to fit each gas reading into the model $Y = b\ (1-e^{-ct})$. gas kinetics were fitted to an exponential model to estimate potential gas production (b) and rate (c). Graphs were generated using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA).

Although the experimental design followed a 3 × 3 factorial arrangement (feed type × fermentation period), the data were analyzed separately for each fermentation time using one-way analysis of variance (ANOVA). This analytical approach was adopted to assess the effect of feed type within each fermentation period independently. Therefore, interactions between feed type and fermentation period were not statistically evaluated, and the results should be interpreted accordingly.

RESULTS AND DISCUSSION

Proximate and fibre composition of the three feed substrates before and after fermentation

Table 1 shows the proximate and fibre composition of rice bran, rice husk, and maize cob at different fermentation periods.

Significant differences ($P < 0.05$) in chemical composition were observed among substrates within fermentation periods, except for acid detergent lignin (ADL) at day 0, crude fibre (CF) at day 30, and ADL at day 45, where differences were not significant. Crude protein (CP) and ether extract (EE) contents differed significantly among substrates at days 30 and 45. Dry matter (DM), ash, CF, neutral detergent fibre (NDF), acid detergent fibre (ADF), and ADL also showed significant variation ($P < 0.05$) among substrates within fermentation periods, indicating differential modification of these components among RB, RH, and MC during fermentation.

The CP content of RB increased from 7.07% at day 0 to 9.44% at day 30, before slightly declining to 8.10% at day 45. This aligns with Christ-Ribeiro et al. (2017), who reported increased CP in rice bran after fermentation with *Rhizopus oryzae*, though differences in fungal strains and fermentation duration may explain variations. Maize cob showed a significant decrease in CF after fermentation, consistent with findings of Akinfemi (2010), Abdel-Aziz et al. (2015), and Ibhaze et al. (2022). The CP content of Rice husk increased from 2.25% to 4.69% by day 45, with concomitant decreases in CF, NDF, ADF and ADL, reflecting trends reported by Dairo et al. (2017) and Sjofjan et al. (2020). Maize cob CP content increased from 4.45% at day 0 to 12.19% at day 45, in agreement with Ibhaze et al. (2022). Variations in literature are attributed to differences in fermentation period, microbial strains, and initial substrate composition (Zaid and Oyedokun Ganiyat, 2009; Oliveira et al., 2010; Omarini et al., 2019). Overall, the fermentation process enhanced the nutritional quality of all three substrates, particularly rice husk and maize cob, by increasing CP and EE and reducing fiber fractions. These changes suggest improved digestibility and potential suitability as ruminant feed, consistent with previous studies (Belewu & Babalola, 2009; Adamafio et al., 2010; Akinfemi, 2010; Yafetto et al., 2023).

Table 1 - Proximate and fibre composition of the three feed substrates at different fermentation periods.

PARAMETERS	DAY 0			SEM	P-value	DAY 30			SEM	P-value	DAY 45			SEM	P-value
	CRB	CRH	CMC			RB:30	RH:30	MC:30			RB:45	RH:45	MC:45		
DM (%)	85.30^{ab}	90.06^{a}	80.76^{b}	1.82	0.01	81.81^{a}	80.98^{a}	71.25^{b}	2.19	0.01	78.94^{a}	73.55^{b}	67.47^{c}	2.01	<0.001
EE (%)	6.90^{ab}	8.39^{a}	5.21^{b}	0.62	0.02	8.55^{b}	11.25^{a}	8.70^{b}	0.59	0.04	15.39^{a}	13.76^{a}	7.65^{b}	1.47	<0.001
ASH (%)	12.82^{b}	18.39^{a}	2.11^{c}	3.01	0.00	11.74^{b}	16.29^{a}	1.81^{c}	2.71	0.00	12.25^{b}	17.14^{a}	1.16^{c}	3.02	<0.001
CF (%)	25.26^{a}	27.04^{a}	30.78^{b}	1.07	0.01	20.51	21.25	20.21	0.35	0.60	9.06^{c}	10.08^{b}	12.66^{a}	0.67	<0.001
CP (%)	7.07^{a}	2.25^{c}	4.45^{b}	0.89	0.01	9.44^{a}	3.07^{c}	5.32^{b}	1.18	0	8.10^{b}	4.69^{c}	12.19^{a}	1.01	<0.001
NDF (%)	48.07^{b}	49.49^{b}	84.72^{a}	7.57	0	38.26^{b}	41.40^{b}	61.53^{a}	4.61	0	46.26^{a}	30.74^{b}	26.30^{c}	3.87	<0.001
ADF (%)	32.01^{b}	31.43^{b}	42.55^{a}	2.32	0	20.23^{b}	21.84^{b}	39.81^{a}	4.01	0	32.00^{b}	41.76^{a}	22.28^{c}	3.55	<0.001
ADL (%)	4.63	4.28	4.00	0.23	0.61	2.38^{b}	2.22^{b}	3.60^{a}	0.28	0.01	1.40	1.60	1.80	0.14	0.62

^{abcd}: Means in the same row with different superscripts are significantly different (P<0.05); NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin, CRB: Rice bran at day zero; CRH: Rice husk at day zero; CMC: Maize cob at day zero; 30RB: Rice bran at 30 days fermentation; 30RH: Rice husk at 30 days fermentation; 30MC: Maize cob at 30 days fermentation; 45RB: Rice bran at 45 days fermentation ; 45RH: Rice husk at 45 days fermentation; 45MC: Maize cob at 45 fermentation

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Effects of fermentation periods on the gas production profile of the substrates

Figure 1 illustrates the cumulative gas production for the substrates at 0, 30, and 45 days of fermentation. Rice bran consistently produced the highest gas volumes at 0 days, indicating higher digestibility of unfermented material. Rice husk showed the lowest gas production, reflecting its high lignin and silica content. Maize cob demonstrated intermediate values.

After 30 days of fermentation, gas production patterns shifted: maize cob and rice husk showed improved cumulative gas production, while rice bran decreased slightly, likely due to depletion of fermentable carbohydrates by fungal activity. The high rate of gas production within 60 to 96 hours, from 4.75 ml to 17.5 ml, indicates that the structure of rice husk might have changed during SSF treatment with enhanced accessibility to the microbes present in the rumen. This improvement corresponded with the results of Tuyen et al. (2013), who established that fungal treatment of rice by-products enhances the ability to cleave lignocellulosic bonds, thereby increasing fermentable carbohydrate yield. The improvements in the cumulative gas production of maize cob are consistent with the work done by Akinfemi et al. (2010), who reported enhanced *in vitro* digestibility of maize cob after fungal pre-treatment. By 45 days, maize cob exhibited the largest increase in gas production (up to 53.03%), suggesting substantial lignocellulosic breakdown and improved fermentability. Rice husk showed moderate improvement (16.10%), and rice bran a smaller increase (21.45%).

Compared with the 30-day fermentation, the 45-day fermentation of rice husk exhibited a completely different pattern. The gas production started slowly at the beginning, but gradually increased over the 96 hours. The increase in gas production from 12 hours to 24 hours ranged from 2.75 ml to 5.25 ml, and this indicates that the longer fermentation time has produced a substrate that is highly fermentable and the rumen microbes have adapted to. The sustained high rate of gas production up to 96 hours suggests that the 45 days of fermentation have converted rice husk into a slowly degradable yet sustained fermentable substrate, which is in support of the findings by Tuyen et al. (2013), who stated that increased fungal treatment of rice straw resulted in enhanced degradation of cell wall components.

The results clearly indicate that a 45-day fermentation period enhanced the digestibility of maize cob, as evidenced by higher gas production compared to both the control and the 30-day fermentation. Gas production at 6 hours (3.25 ml) was equivalent to that of the control at 12 hours, suggesting that fermentation made nutrients more readily available for rapid microbial utilization. This observation is consistent with Akinfemi et al. (2010), who reported higher soluble carbohydrate content in fungal-treated maize cob.

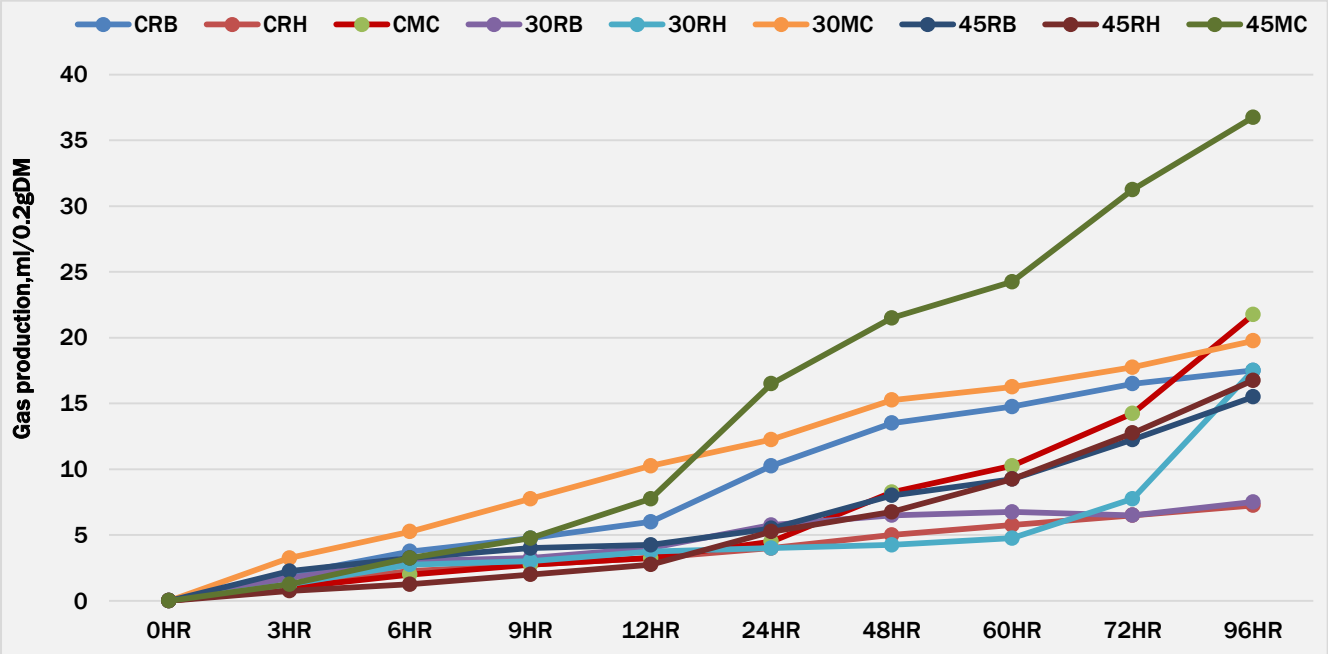

Figure 3 - In vitro gas production of the rice bran, rice husk and maize cob at 0, 30 and 45 days of fermentation. Data points represent mean volumes (ml) of triplicate samples per 0.2 g DM. CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation); 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

Gas production kinetics of the substrates at different fermentation periods

Results of gas production kinetics of the feed types over the fermentation days are shown in Table 2. Kinetics of gas production describe the amount of gas that a material produces (b), and 'c' is the rate at which these gases are produced. These parameters show how digestible a feeding material is and how long it would remain in the gut (Osafo et al., 2023). The results indicate that at day 0, Maize cob (MC) had the highest gas production (19.55%), suggesting it contains a higher proportion of fermentable carbohydrates. Rice husk (RH) had the lowest (6.44%), likely due to its high

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lignin and silica content, which limits microbial degradability. After the 30 days of fermentation, maize cob recorded 17.81%, and rice husk value was 16.21%, showing significant improvement in gas production, likely due to the breakdown of lignocellulosic structures by fungi. After 45 days of fermentation, the Maize cob recorded a value of 53.03%, which had an extremely high increase, indicating enhanced delignification and availability of fermentable substrates. Rice husk also recorded 16.10%, showing improvement, but remained lower, probably due to persistent high fiber content. Thus, gas production potential (b) significantly increased ($P<0.05$) for all substrates after 45 days of fermentation, particularly for maize cob, which showed a marked increase from 19.55% to 53.03%. Such an enhancement suggests that the fermentative treatment substantially improves the availability of fermentable carbohydrates and overall substrate digestibility.

This observation is consistent with reports demonstrating improved substrate breakdown through microbial action during extended fermentation periods (Asiegbu et al., 1994). In the case of rice bran, a moderate improvement from 17.68% to 21.44% in gas potential was observed, which aligns with studies showing that fermentation can enhance the nutritional quality and digestibility of rice bran by modifying its carbohydrate profile and reducing anti-nutritional factors (Widiyastuti et al., 2019). Conversely, rice husk, which initially had a lower gas production potential (6.44%), exhibited an increase to 16.10% following longer fermentation. This improvement is attributable to the microbial degradation of lignocellulosic components, rendering the substrate more accessible to ruminal microbes (Fapohunda et al., 2013)

The rate of gas production (c hr^{-1}) showed no significant differences among most treatments, indicating that the fungal fermentation primarily influenced the extent rather than the speed of fermentation. From Table 2, it can be observed that rice bran's rate decreased from 0.03 hr^{-1} to 0.01 hr^{-1} after extended fermentation. This reduction likely reflects a shift from rapid fermentation of readily available carbohydrates to a slower fermentation phase as the easily fermentable components are depleted, a phenomenon noted in kinetic studies of ruminal fermentation (Widiyastuti et al., 2019). For rice husk, an increase in the rate from 0.03 hr^{-1} to 0.08 hr^{-1} was observed, indicating that fermentation may have enhanced the accessibility of fiber constituents, thereby accelerating initial microbial action (Fapohunda et al., 2013). In contrast, maize cob's rate decreased significantly from 0.08 hr^{-1} to 0.01 hr^{-1}, suggesting that while the overall extent of fermentation was improved (as shown by the increased gas production potential), the fermentation kinetics shifted toward a slower phase once the rapidly fermentable fractions were utilized (Asiegbu et al., 1994).

Estimated digestibility and energy parameters of the substrates at different fermentation periods

Table 3 shows the results of the *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), net energy (NE), short-chain fatty acids (SCFA) and microbial protein (MP) values for the substrates. Rice bran (RB) at day zero had the highest digestibility and metabolizable energy values (IVOMD, ME) as well as SCFA and MP yield. Rice husk (RH) and maize cob (MC) had similar values for IVOMD, ME, NE and MP. After solid-state fermentation (SSF) with *Pleurotus ostreatus*, maize cob showed the largest gains: IVOMD significantly increased from 21.01 at day zero to 34.74 g/kg DM by day 45 and the energy values (ME and NE) also showed a substantial improvement. In contrast, RB's digestibility and energy slightly decline with SSF, while RH shows modest improvement over longer fermentation.

A study by Espinosa-Páez et al. (2017) confirms that SSF using *Pleurotus ostreatus* tends to increase crude protein and metabolizable energy but can reduce fiber and even lower digestibility if excessive. The present study was consistent with the study by Nasehi et al. (2016), which found that SSF of crop by-products (including rice husk) raised CP, ash, ME, and NEL in all substrates;however, IVOMD and SCFA gains were lower for rice husk (there was no significant change in husk digestibility or SCFA). Conversely, according to Ikwunze et al. (2024) *Pleurotus*-treated maize cob significantly improved OMD and SCFA, with OMD and fermentable fraction increasing as the number of days increased. These findings align with the present study, which showed that the digestibility (IVOMD) and energy contents of MC improved significantly after SSF using *Pleurotus ostreatus*. The MP values increased from 4.15 at day zero to 6.79 at day 45.

Rice bran at day zero showed the highest IVOMD and ME values of 27.82 g/kg and 3.98 MJ/kg, respectively, but these declined to 24.4 g/kg and 3.57 MJ/kg at day 45. This reduction aligns with the study of Sánchez et al. (2002) who explained that prolonged fermentation periods can result in a reduction of fat and fiber fractions, alongside notable increases in protein and ash content. Thus, the RB fermented in the current study had more fungal protein but lost some soluble nutrients, lowering its IVOMD and ME (Ibarruri et al., 2021). Rice bran had the lowest IVOMD value of 20.74 % at day zero due to the presence of lignin and silica. The IVOMD value increased to 22.89 at day 45. The SCFA content also followed the same trend. Studies by Nasehi et al. (2016) using *Pleurotus. florida* for solid-state fermentation on rice husk led to similar, results with no substantial improvement in rice husk digestibility and SCFA production. The total metabolic energy of rice husk increased slightly from 2.91 to 3.32 MJ/kg during the fermentation process.

The ME content 2.99 MJ/kg of MC at day zero increased to 5.03 MJ/kg at day 45. Sufyan et al. (2021) screened *P. ostreatus, Pleurotus eryngii and Pleurotus florida* using corn cob and found that *Pleurotus florida* gave the largest lignin degradation and digestibility. Ikwunze et al. (2024) in their research found that *Pleurotus* tuber-regium-treated maize cob significantly increased *in vitro* digestibility, gas production, SCFA and metabolizable energy as fungal content increased. Akinfemi (2010) similarly reported *Pleurotus ostreatus* raised maize cob CP (3.89 to 10.11%) and OMD (control 48.32% to 60.75%), with ME up to 8.59 MJ/kg.

Table 2a - Gas production kinetics of the substrates at different fermentation periods.

Parameters	Feed substrate (Day 0)			SEM	P-values	30 days fermentation			SEM	P-Values
	CRB	CRH	CMC			RB:30	RH:30	MC:30		
b (%)	17.68[a]	6.44[b]	19.55[a]	2.60	<0.001	6.87[b]	16.21[a]	17.81[a]	2.16	<0.001
c (hr^{-1})	0.03	0.03	0.08	0.029	0.07	0.05	0.07	0.06	0.01	0.78

[abcd] Means found in the same row with different superscripts are significantly different (P<0.05); CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation); 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

Table 2b - Gas production kinetics of the substrates at different fermentation periods.

Variables	45 days fermentation			SEM	P-Values	30 and 45 fermentations (Interaction)						SEM	P-Values
	RB:45	RH:45	MC:45			RB:30	RH:30	MC:30	RB:45	RH:45	MC:45		
b (%)	21.45[b]	16.10[b]	53.03[a]	7.38	0.004	6.87[c]	16.21[b]	17.81[b]	21.45[b]	16.10[b]	53.03[a]	4.43	<0.001
c (hr^{-1})	0.01[b]	0.08[a]	0.01[b]	0.01	0.001	0.05	0.07	0.06	0.01	0.08	0.01	0.01	0.07

[abcd] Means found in the same row with different superscripts are significantly different (P<0.05); CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation); 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

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Table 3a - Estimated digestibility and energy parameters of the substrates at different fermentation periods.

Parameters	Feed substrate (Day 0)			SEM	P-values	30 days fermentation			SEM	P-Values
	CRB	CRH	CMC			RB:30	RH:30	MC:30		
IVOMD- (%)	27.82^{a}	20.74^{b}	21.01^{b}	1.47	<0.001	25.15^{a}	20.94^{c}	22.98^{b}	0.77	<0.001
ME (MJ/kg DM)	3.98^{a}	2.91^{b}	2.99^{b}	0.22	0.001	3.61	2.99	3.51	0.13	0.055
NE (MJ/kg DM)	3.00^{a}	2.62^{a}	2.57^{a}	0.09	0.047	3.05	2.70	2.83	0.07	1.117
SCFA	21.85^{a}	8.55^{b}	9.79^{b}	2.68	<0.001	12.38^{a}	8.59^{b}	13.60^{a}	0.96	<0.001
MP	5.49^{a}	4.10^{b}	4.15^{b}	0.29	0.004	4.92^{a}	4.13^{ab}	4.58^{a}	0.15	0.035

^{abcd} Means found in the same row with different superscripts are significantly different (P<0.05); IVOMD: *In vitro* organic matter digestibility, MP: Microbial protein, ME: metabolizable energy, SCFA: Short chain fatty acids, NE: Net energy; CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation); 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

Table 3b - Estimated digestibility and energy parameters of the substrates at different fermentation periods.

Variables	45 days fermentation			SEM	P-Values	30 and 45 fermentations (Interaction)						SEM	P-Values
	RB:45	RH:45	MC:45			RB:30	RH:30	MC:30	RB:45	RH:45	MC:45		
IVOMD (g/kg DM)	24.34^{b}	22.89^{b}	34.74^{a}	2.36	<0.001	25.15^{b}	20.9^{e}	22.98^{d}	24.3^{c}	22.89^{d}	34.74^{a}	1.35	<0.001
ME (MJ/kg DM)	3.57^{b}	3.32^{b}	5.03^{a}	0.34	0.004	3.61^{b}	2.99^{b}	3.51^{b}	3.57	3.32^{b}	5.03^{a}	0.19	<0.001
NE (MJ/kg DM)	3.15	2.84	3.60	0.16	0.091	3.05^{a}	2.70^{b}	2.83^{b}	3.15^{a}	2.84^{b}	3.60^{a}	0.09	0.019
SCFA	11.87^{b}	11.37^{b}	35.25^{a}	4.98	<0.001	12.38^{c}	8.59^{e}	13.60^{b}	11.87^{cd}	11.37^{d}	35.25^{a}	2.70	<0.001
MP	4.77^{b}	4.55^{b}	6.79^{a}	0.45	0.002	4.92^{b}	4.13^{c}	4.58^{b}	4.77^{b}	4.55^{b}	6.79^{a}	0.26	<0.001

^{abcd} Means found in the same row with different superscripts are significantly different (P<0.05); IVOMD: *In vitro* organic matter digestibility, MP: Microbial protein, ME: metabolizable energy, SCFA: Short chain fatty acids, NE: Net energy; CRB: Rice bran at day zero; 30RB: Rice bran at 30 days fermentation; 45RB: Rice bran at 45 days fermentation, CRH: Rice husk at day zero; 30RH: Rice husk at 30 days fermentation); 45RH: Rice husk at 45 days fermentation; CMC: Maize cob at day zero; 30MC: Maize cob at 30 days fermentation; 45MC: Maize cob at 45 days fermentation.

CONCLUSION AND RECOMMENDATION

Solid-state fermentation (SSF) using *Pleurotus ostreatus* improved the nutritional quality and *in vitro* fermentation characteristics of rice bran, rice husk, and maize cob. The process improved crude protein content and reduced the content of the detergent fibre fractions, resulting in enhanced fermentability and *in vitro* digestibility, especially in maize cob and rice husk. Upon treatment, *In vitro* organic matter digestibility, metabolizable energy, short-chain fatty acids, Net energy, and Microbial protein were also improved. The study confirms that SSF using *Pleurotus ostreatus* is a promising technique for upgrading low-quality agro-by-products into more digestible and nutritionally valuable ruminant feed resources.

The findings of this study should be interpreted considering the analytical limitations. Improvements observed in nutritional and fermentation characteristics should be specified according to the particular substrate and fermentation period in which they occurred. Future research should employ a factorial analysis to investigate potential interactions between feed type and fermentation duration, which would provide a more comprehensive understanding of their combined effects. In addition, *in vivo* trials with ruminant animals are recommended to validate the in vitro results and to evaluate animal performance indicators such as feed intake, weight gain, milk yield, and feed conversion efficiency.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dennis Afram; E-mail: aframdennis1@gmail.com; ORCID: 0009-0000-4421-4877

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

D. AFRAM contributed to the design of the experiment, data collection, statistical analyses and interpretations, and the writing of the manuscript. V. ATTOH-KOTOKU made significant contributions to the conceptualization and experimental design, was involved in the interpretation of results, contributed to both drafting and revision of the manuscript, and supervised the study. E.E.T.GYAMFI participated in data collection, interpretation of results and manuscript drafting. J.VAN-ESS participated in data collection, interpretation of results and manuscript drafting. N.WILLIAMS participated in data collection, interpretation of results and manuscript drafting, A.F.ABOAGYE participated in data collection, interpretation of results and manuscript drafting. N.WILLIAMS participated in data collection, interpretation of results and manuscript drafting. A. DONKOH was involved in the interpretation of results and the manuscript preparation. P. SASU participated in the interpretation of results and facilitated the drafting and revision of the manuscript. K.G. OWUSU engaged in data collection, statistical analysis, and manuscript drafting. B.ADJEI-MENSAH participated in the interpretation of results and the drafting of the manuscript.

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Competing interests

The authors declare no competing interests in this research and publication.

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