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Pleurotus ostreatus Can Significantly Improve the Nutritive Value of Lignocellulosic Crop Residues

Lydia K. Olagunju [1], Omoanghe S. Isikhuemhen [2,*], Peter A. Dele [1], Felicia N. Anike [2], Brandon G. Essick [2], Nathan Holt [2], Nkese S. Udombang [2], Kelechi A. Ike [1], Yasmine Shaw [1], Rosetta M. Brice [1], Oluteru E. Orimaye [1], Michael Wuaku [1] and Uchenna Y. Anele [1,*]

[1] Department of Animal Sciences, North Carolina Agricultural and Technical State University, 1601 East Market Street, Greensboro, NC 27411, USA; lkolagunju@aggies.ncat.edu (L.K.O.); padele@ncat.edu (P.A.D.); kaike@aggies.ncat.edu (K.A.I.); rosettab101@gmail.com (R.M.B.); oeorimaye@aggies.ncat.edu (O.E.O.); mwuaku@aggies.ncat.edu (M.W.)

[2] Department of Natural Resources and Environmental Design, North Carolina Agricultural and Technical State University, 1601 East Market Street, Greensboro, NC 27411, USA; fnanike@ncat.edu (F.N.A.); bgessick@ncat.edu (B.G.E.); naholt@ncat.edu (N.H.); nsudombang@aggies.ncat.edu (N.S.U.)

* Correspondence: omon@ncat.edu (O.S.I.); uyanele@ncat.edu (U.Y.A.)

Abstract: Improvement in the nutritive value of corn stover after solid-state fermentation with *Pleurotus ostreatus* is reported. Two ruminally cannulated dairy cows were used in an in vitro study arranged in a 2 × 3 factorial design with four replicates using *P. ostreatus*-treated corn stover. The increase in crude protein (58.5%) and ash (25.8%) contents of the treated stover were significantly higher ($p < 0.001$) than the control. Results suggest a potential increase in in vitro microbial protein synthesis due to a consistent increase ($p < 0.001$) in microbial mass yield (106–681%), irrespective of the incubation time. The fiber components of the corn stover, except lignin, were significantly reduced ($p < 0.05$), and the non-fiber carbohydrates were increased by 118% in the treated samples. There was a significant ($p < 0.001$) increase in both in vitro dry matter disappearance (40.9–240%) and total VFA (5.85–11.2%). Treatment and time interaction was significant ($p < 0.001$) for propionate production (9.37–14.0%), indicating that the propionate rumen fermentation pathway was preferred. Acetate concentration was reduced (1.76–4.01%), which also resulted in a 7.09–11.42% decrease in the A:P ratio. Overall, results showed that *P. ostreatus* improved the nutritive value of corn stover by increasing its energetic values, crude protein, microbial mass, and total volatile fatty acid concentrations.

Keywords: corn stover; bioconversion; feedstuff; nutritive value; *Pleurotus ostreatus*

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1. Introduction

Livestock production plays a critical role in food security worldwide as demand always exceeds supply [1]. With the world's population projected to reach 9.6 billion by 2050 [2], there will be a concomitant increase (projected to increase by 60 to 70%) in demand for animal products [3] and these projections have created an awareness of food security for the fast-growing human population [4]. Historically, feed has been noted as the main driver in livestock production, and the single largest variable input cost as it accounts for 60 to 70% of production cost [3,5,6]. Forage is the major and most economical source of energy for ruminants and a significant component of their diet, which ranges from 40 to 100% [7–9]. Yet, forage is composed of fiber, which limits its digestibility since fiber consists of structural carbohydrates [10,11], such as hemicellulose, and cellulose, complexed with lignin, which is generally known as the primary component of forage that limits the digestion of forage [7,12].

Therefore, research to improve the quality and availability of feed resources was cited as crucial for increasing production efficiency. Although using novel feeds to provide alternative sources was suggested [13], the potential of such novel feeds will need to be

confirmed. However, studies suggest that harnessing agricultural wastes could be a reliable source of cheap feed for increased productivity [14,15]. More so, agricultural by-products have been reported to be abundantly available worldwide [16–18]. Agricultural wastes generated annually are estimated at 998 metric tons globally [19]. Corn stover is reported to be in abundance and underutilized as animal feed due to the inherent challenges of high fiber content [20], thus regarded as lignocellulosic biomass with a low nutrient profile. Therefore, any practical interventions or technology for the delignification of lignocellulosic biomass to increase its digestibility are welcome. Though there are physical and chemical treatments to disrupt carbohydrate–lignin complexes in lignocellulosic crop residues to improve their feeding value [21–23], their practical application is limited due to societal and environmental concerns. Additionally, the use of microbial additives to promote digestion, intestinal hygiene, and animal performance was reported to be widely investigated [14] which has resulted in the acceptance of biological treatment as an alternative practical and environmentally friendly approach to enhance the nutritive value of corn stover [14,18].

Asmare [14] provided extensive information on studies conducted on the biological treatment of crop residues with white rot fungi that yielded positive results. It was reported that biological treatment can break lignocellulose structure to liberate cellulose and improve digestibility and nutrients, thus enhancing the feeding value for increased animal production. Previous studies have shown that white rot treatment of different crop residues resulted in significant changes in the lignin, neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents with values ranging from lower than 10% and as high as over 70% [7,14,18]. Other authors reported a knowledge gap in the approaches for corn stover treatments to improve digestibility [18,24].

Biological agents such as fungi were reported to have extracellular enzymes such as laccase, manganese peroxidase, and lignin peroxidase that can degrade lignin in cell walls, and white rot species were identified to be suitable with no side effects for optimal improvement of crop residues [25]. Moreover, white rot fungus was reported to degrade lignin to a great extent and at a fast pace when compared with any other group of organisms [14,26]. Therefore, it was recommended as a potential solution to remove lignin and/or increase the digestibility of low-quality lignocellulosic crop residues, but its utilization was noted to be hindered by the lack of knowledge and technology.

Pleurotus species has been proven to be more efficient among the edible white rot fungi in recycling agricultural waste and generating bioactives with medicinal properties [21,23,27–32]. We hypothesize that fermentation with *P. ostreatus* will achieve delignification and improve the nutritive value of corn stover. Therefore, the study aimed to demonstrate, in vitro, the ability of solid-state fermentation (SSF) with *P. ostreatus* to improve the nutritive and feed value of corn stover.

2. Materials and Methods

2.1. Solid-State Fermentation

Twenty replicate bags, each weighing 2 kg were prepared by using shredded (5 cm in length) corn stover substrate, after soaking in water for 18 h, and drained for 12 h. The bags were sterilized (121 °C, 15 psi for 1 h). Upon cooling, 16 of the bags were inoculated with *P. ostreatus* grain spawn (5% wet weight) and incubated at 25 °C. Four bags were dried at 60 °C for 48 h and it was used as the control. Sampling was conducted after 3 weeks of incubation, dried as above, and milled to pass through 1 mm sieve size before use for in vitro studies.

2.2. Animal Care and Feeding

All animal procedures and uses were approved by the North Carolina Agricultural and Technical State University Institutional Animal Care and Use Committee. This study was conducted at North Carolina Agricultural and Technical State University Dairy Research and Training Facility (NCAT DRTF; Greensboro, NC, USA). The cannulated cows were

observed daily for health problems and treated according to routine management practices at the DRTF maintained under IACUC-approved protocol LA21-009.

2.3. Experimental Design

The study was a 2 × 3 factorial design with 2 treatments and 3 incubation periods. The treatments were treated and untreated corn stover was analyzed for chemical composition and evaluated in an in vitro batch culture technique at 6, 24, and 48 h periods. A total of four replicates were prepared for each treatment for the various periods.

2.4. Sample Preparation

Treated or untreated corn stover, approximately 0.5 g each was weighed with an analytical scale (model VWR-224AC; VWR International, Radnor, PA, USA) directly into 100 mL serum bottles (Cat# 223747; Wheaton Science Products, Millville, NJ, USA). There were four replicates (bottles) prepared for each treatment and incubated for 6, 24, and 48 h. To determine digestibility, 0.5 g of the samples were weighed into Ankom bags (F57; Ankom Technology Corp, Macedon, NY, USA) and sealed using a heat impulse sealer (Model # AIE-200HR, California, USA), which were inserted into pre-labeled 100 mL serum bottles according to treatment with four replicates per treatment.

2.5. In Vitro Batch Culture

The in vitro batch fermentation experiments were repeated two times on different days using rumen fluid sourced from the same cannulated cows. Rumen fluid (RF) was sampled from the cranial, ventral, and dorsal regions of the rumen. Thereafter, the solid part of the rumen fluid was filtered out using four layers of cheesecloth. The liquid part was funneled into a pre-warmed insulated thermoflask. Rumen fluid was collected via the rumen cannula at approximately 4 h after feeding from two multiparous mid-late lactations ruminally cannulated grazing Holstein cows. During the trial, cows averaged 705.9 ± 78.1 kg of BW, 120 ± 91.1 days in milk, and 31 ± 15.5 kg/d of milk yield. Cows were milked twice per day at 04:00 and 16:00 h with ad libitum access to water and are supplemented with a total mixed ration containing 20% corn silage, 75% concentrate ration, and 5% mineral and vitamin supplement (on dry matter (DM) basis).

The in vitro procedure was based on the methods described by [33], with some modifications. The RF from the 2 individual cows was mixed and strained through 4 layers of cheesecloth and transported immediately to the lab in a Thermo flask. The pH of the pooled RF sample was measured immediately using a benchtop pH meter (model B10P; VWR International, Radnor, PA, USA). Aliquots of 15 mL of strained RF anaerobically flushed with CO_2 and maintained at 39 °C in a water bath (model WBE28; VWR International, Radnor, PA, USA) were added into the 100 mL serum bottles (Wheaton Science Products, Millville, NJ, USA) containing a previously CO_2 gassed 45 mL of McDougall's buffer and pre-warmed at 39 °C in a water bath (model JAB18; Grant Instruments Ltd., Cambridge, UK). Bottles were capped with 20 mm rubber stopper (Wheaton Science Products, Millville, NJ, USA) and crimped with 20 mm aluminum caps (Wheaton Science Products, Millville, NJ, USA). The in vitro batch fermentation was performed in a reach-in incubator (model 1915A; VWR International, Radnor, PA, USA) at 39 °C for 6, 24, and 48 h with an orbital shaker (model 3500; VWR International, Radnor, PA, USA) at a speed of 125 rpm. Six blanks were included for the digestibility study and four contained only RF and buffer (without bags) to correct for background gas production and two contained one bag each (without sample) placed in the serum bottle for correction factor and the blanks were prepared for each time period according to experimental design. Four blanks were included for the microbial mass study containing only RF and buffer (without samples).

Gas production was measured as pressure per square inch (PSI) in each bottle at each time period by inserting a BD 0.7 mm × 40 mm (Cat# 301000) hypodermic gauge needle attached to a manometer (model 33500-086; VWR International, Radnor, PA, USA). Once the 6, 24, and 48 h time periods were completed, the serum bottles were decapped

with a decapping plier (Cat# C4020-101; Thermo Scientific, Rockwood, TN, USA), and the Ankom bags were washed with cold water and oven dried at 55 °C for 48 h. The bags were then placed in a desiccator for 20 min and then weighed for dry matter disappearance and other digestibility analyses. The bags were stored for further fiber analysis. Fifteen milliliters of RF from each bottle were pipetted into a vial containing three milliliters of 25% metaphosphoric–crotonic acid and stored at −20 °C for volatile fatty acid (VFA) concentration. The inoculum for the microbial mass study was transferred into pre-weighed 50 mL bottles (Thermo Scientific Nalgene Products; Rochester, NY, USA) and centrifuged using Thermo Fisher Scientific centrifuge (model SORVALL RC-6 plus; Thermo Fisher Scientific Inc., Asheville, NC, USA) at 10,000× *g* for 15 min. The pellets were harvested and frozen at −20 °C. In vitro apparent digestibility (IVADDM) and in vitro true digestibility (IVTDDM) were estimated as previously described by [33].

2.6. Chemical Analysis

Ground samples were weighed and analyzed for C, H, and N using the Classical Pregl–Dumas method [34]. Samples were analyzed for nitrogen content via the pure combustion method (Pregl–Dumas method) using PerkinElmer CHN/O analyzer (model 2400 Series II; PerkinElmer Inc., Waltham, MA, USA). Nitrogen content was then multiplied by 6.25 to calculate crude protein (CP). Ash content was determined by incinerating a 2 g sample for 3 h at 550 °C in a muffle furnace (model BF51728C-1; Thermo Fisher Scientific, Asheville, NC, USA) using [35]. Organic matter (OM) was calculated as 100 − ash. Petroleum ether was used to determine ether extract (EE; AOCS Standard Procedure Am 5-04) [36] in an Ankom XT15 fat extractor system (Ankom Technology Corp., Fairport, NY, USA). Non-fibrous carbohydrate was calculated as NFC (%) = 100 − (% Ash + % CP + % NDF + % EE) according to [37]. Subsamples of treated and untreated corn stover, and incubation residues were oven-dried at 55 °C for 48 h to determine DM. The analysis of NDF was conducted using the filter bag technique method [38] with a heat-stable alpha-amylase and sodium sulfite (Ankom Technology; Macedon, NY, USA). The ADF [39] was analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY, USA), and acid detergent lignin (ADL) was determined using 72% sulfuric acid (H_2SO_4) in a beaker for 3 h. Hemicellulose content is calculated from the difference between NDF and ADF, and cellulose from the difference between ADF and ADL.

2.7. Microbial Mass

Microbial mass determination was based on [40] with a slight modification. The samples were freeze-dried for 96 h using BUCHI freeze dryer (Model DUO 6 M; BUCHI Labortechnik AG, Flawil, Switzerland). The freeze-dried samples were weighed with an analytical scale (model VWR-224AC; VWR International, Radnor, PA, USA) to determine the difference in weight before and after freeze-drying. The microbial mass was calculated as: Feed (DM) incubated − [pellet (DM) − blank pellet (DM)]/Feed (DM) incubated. The partitioning factor (PF) was calculated as the ratio of mg substrate truly degraded/mL gas produced according to [41].

2.8. Volatile Fatty Acid

Rumen fluid samples that were preserved with 25% metaphosphoric–crotonic acid were thawed and centrifuged at 10,000× *g* for 15 min at 4 °C using a Thermo Fisher Scientific centrifuge (model Sorvall X4R Pro-MD; Thermo Electron LED GmbH, Osterode, Germany) and analyzed according to the protocol in [42] for acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations using an automated GC (model 7890 B; Agilent Technologies, Santa Clara, CA, USA) and a flame ionization detector. Volatile fatty acids were separated on a capillary column ZB FFAP (30 m × 0.25 mm i.d., 0.25 um; Phenomenex Inc., Torrance, CA, USA) using a metaphosphoric–crotonic acid mixture as an internal standard. The split ratio of 1:12 in the injector port was at a temperature of 250 °C with a flow rate of 1 mL/min of helium. The oven temperature was initially set

at 120 °C for 0.8 min; it then increased by 8 degrees per minute until it reached 140 °C. The detector temperature was maintained at 280 °C.

2.9. Estimated Parameters

The estimated parameters were calculated using the following formulae:

Dry matter intake (DMI) = 120/NDF, dry matter digestibility (DDM) = 88.9 − (0.779 × ADF), relative forage value (RFV) = (DDM × DMI) × 0.775, relative forage quality (RFQ) = (DMI × TDN)/1.23, total digestible nutrient (TDN) = 104.97 − (1.302 × ADF), digestible crude protein (DCP) = (0.916 × CP) − 3.09, gross energy (GE) = (CP × 0.056) + (EE × 0.094) + (100 − CP − Ash − EE) × 0.042, digestible energy (DE) = TDN × 0.04409, metabolizable energy (ME) = 0.821 × DE, net energy of maintenance (NEM) = (TDN × 0.029) − 0.29, net energy of growth (NEG) = (TDN × 0.029) − 1.01, net energy of lactation (NEL) = (TDN × 0.0245) − 0.012 [43–47].

2.10. Statistical Analysis

Data were analyzed as repeated measures using the MIXED procedure of SAS software [48]. The MIXED model accounted for the repeated measures (sampling day), the fixed effect of treatment. For the repeated measures, various covariance structures were tried with the final choice depending on low values from the Akaike's information criteria (AIC), which were compared using Duncan's multiple comparisons. The model used was:

$$Yijk = \mu + Ti + Pj + (TP)ij + Eijk,$$

where Yijk is each individual observation for a given variable, μ is the overall mean, Ti is the treatment effect, Pj is the incubation period effect, (TP)ij is the interaction between treatment and period, and Eijk is the residual error. Significance was declared at $p \leq 0.05$ and a trend at $0.05 < p \leq 0.10$ unless otherwise stated.

3. Results and Discussion

3.1. Chemical Composition

Table 1 The DM content was significantly ($p < 0.001$) influenced by the treatment. The increase of 6.57% in the DM content of the treated samples was driven mainly by increases in their CP and NFC contents. This will result in more nutrients being available for animals in the treated substrate since DM content is an indicator of the amounts of nutrients available in feeds. The current increment in DM showed that mycelium tends to increase nutrient availability while interacting with the substrate du shows the chemical composition of treated and untreated corn stover after a 3-week solid-state fermentation. Changes in the chemical composition of the treated samples confirmed that mycelium secretes an array of compounds into its substrates that can help release bound nutrients, thereby altering the chemical nature of the substrates [49–52].ring degradation to support its growth and this observation is consistent with a previous report by [52]. Contrary to the present study, refs. [18,30,51] reported a decrease in the DM of rice straw, maize stover, and wheat straw treated with *P. ostreatus*. Differences in the strain and incubation temperature could be responsible for the observed changes in the DM content. For example, [18] incubated their substrates at 24 °C compared to 25 °C in the present study and they used 11 strains of white rot fungi (including *P. ostreatus*) in their study.

Table 1. Chemical composition (% DM [1]) of *Pleurotus ostreatus* treated and untreated corn stover (n = 8).

Variable	Untreated	Treated	SEM	p-Values
DM, %	91.4 [b]	97.4 [a]	0.85	<0.001
OM, %	96.3 [a]	95.4 [b]	0.21	0.021
CP, %	3.42 [b]	5.42 [a]	0.301	<0.001
ASH, %	3.69 [b]	4.64 [a]	0.205	0.021
EE, %	8.92 [a]	4.13 [b]	0.484	<0.001
NFC, %	12.6 [b]	27.5 [a]	2.16	<0.001
NDF, %	71.3 [a]	58.3 [b]	1.17	<0.001
ADF, %	44.7 [a]	41.7 [b]	0.57	0.006
HEM, %	26.7 [a]	16.6 [b]	1.45	<0.001
CELL, %	37.4 [a]	34.5 [b]	0.53	0.014
ADL, %	7.25	7.18	0.253	0.899

[1] DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFC, non-fiber carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; HEM, hemicellulose; CELL, cellulose; ADL, acid detergent lignin. [a,b] Means with different superscripts within each row differ by $p < 0.05$.

Ash content increased (p = 0.021) from 3.69% in untreated to 4.64% in treated samples, which resulted in a 25.8% increase but within the range considered to be a normal ash content in forages. This is consistent with a previous report by [53], which indicated that complete mineralization for biodegradation might be quite long, thereby necessitating the need to investigate an appropriate incubation period for optimal mineralization of spent mushroom substrate. Fazaeli et al. [23] reported that an increase in ash content might have a negative impact on organic matter (OM) but our current study showed that OM was only decreased by 0.943%, and not expected to reduce the proportion of available nutrients.

There was a 58.5% increase ($p < 0.001$) in the CP content of the treated samples which could have resulted from the efficient conversion of nitrogen to microbial protein by *P. ostreatus*. Higher CP in the treated corn stover samples is advantageous to the rumen microbes and is expected to improve protein availability to rumen microbes for optimal ruminal efficiency and microbial protein production. Thus, enhancing the feeding value and invariably could provide more essential amino acids to improve animal performance [54]. The increase in CP content in the current study is similar to the 5.08% CP reported [30] on substrates from bioconversion of wheat straw with white rot fungi. Additionally, ref. [14] reported that the increased protein content might be due to the bioconversion of organic materials down to one of the fungal body components or due to the addition of microbial protein, which is considered an important source of daily metabolizable protein for cattle production [55]. Contrary to our study, ref. [56] reported no significant improvement in CP in maize straw treated with *P. osteratus*. Unlike the current study, ref. [56] used two different temperatures and the samples were incubated with and without light. The samples were first incubated without light for 15 d and maintained at a temperature of 25 °C and later exposed to daylight for 8 d and maintained at a temperature of 21 °C.

The ether extract was significantly ($p < 0.001$) lower in the treated compared with the untreated samples, which resulted in a 53.7% decrease in EE concentration. The decrease could have resulted from biologically active phenolic compounds from *P. ostreatus* (e.g., lovastatin) which are known to have strong depressing effects on lipids [57,58]. This could likely favor DM digestibility and microbial growth which are negatively affected by EE [59,60]. The decrease in EE for treated samples is consistent with [51] but [50] reported an increase in EE when rice straw was treated with *P. ostreatus*. It could be that substrate type and source (where cultivated) are driving the differences noted in the EE.

3.2. Loss of Organic Matter

There was a significant ($p < 0.05$) treatment effect on OM content with a negligible decrease of 0.943% in the treated samples. The small decrease in nutrients from the treated corn stover during fungal growth is desirable because more OM would be available for rumen fermentation. It also showed that a low amount of structural carbohydrates was

utilized by *P. ostreatus* to build up microbial mass and degrade lignin [61], resulting in more available nutrients to improve animal performance. The small reduction in OM content in the present study is not consistent with previous reports [18,62–64] and differences in OM could be a result of the differences in the culture condition, strain, incubation period, and temperature.

3.3. Fiber Composition

It is known that the lignocellulosic complex in lignocellulosics can stimulate the lignocellulolytic enzymatic activity of *P. ostreatus* to result in the significant breakdown of the lignocellulosic complex to release inaccessible nutrients [23,24]. Our results indicated that approximately 118% increase ($p < 0.001$) in NFC and the various fiber components, NDF, ADF, and hemicellulose were degraded. After incubation, there were 18.2, 6.71, 37.8, and 7.75% reductions in NDF, ADF, hemicellulose, and cellulose concentrations, respectively, for the treated samples when compared with untreated samples. The impact of *P. ostreatus* on lignin was not significant ($p > 0.05$), probably due to the short period of SSF (3 weeks). However, results confirmed that the carbohydrate lignin complex was disrupted, which will result in an increased rate of passage through the rumen [65].

The insignificant lignin degradation noted in the present study is consistent with some previous studies that reported no significant effect on lignin in *P. ostreatus* wheat straw [50,51]. Another study [64] reported that *P. ostreatus*-treated wheat straw reduced lignin content as the lignin in the wheat straw was degraded during the second phase of fungal growth. Readily soluble components of substrates are typically metabolized for fungal use during the early stages of substrate colonization [64]. This may imply that a longer time for SSF with *P. ostreatus* is necessary to achieve significant lignin degradation in corn stover. Therefore, there is a need to investigate the effect of different incubation periods on lignin degradation in corn stover. The 7.75% reduction in cellulose was in contrast with the report by [18], who reported that *P. ostreatus* had no effect on the cellulose content of maize stover. Additionally, refs. [62] and [66] reported that hemicellulose was utilized more than cellulose by *P. ostreatus*, and cellulose degradation was not detected during the early days of inoculation, which suggests that the incubation period can influence the degradation process. Similarly, ref. [53] noted that degradation of lignocellulose might be associated with mycelial growth habit and this view is supported by [66] who reported that enzyme activities change considerably during the shift between substrate colonization and fructification stages of mushroom growth. In general, the early stages of lignocellulose degradation by white rot fungi do not involve cellulose utilization.

Interactions between treatment and in vitro batch culture fermentation time were significant ($p < 0.001$) for DMD, NDFD, ADFD, ADLD, HEMD, and CELLD. Expectedly, the highest DMD value was noted for the treated samples after 48 h of in vitro fermentation (Table 2) and a similar trend was noted for ADLD concentration. The other fiber component did not follow this trend of increased digestibility with increasing fermentation time. A decrease in NDFD and HEMD digestibility might have resulted from the consumption of easily digestible substances present in the substrate by the other microbes [64]. Both NDFD and HEMD were highest in the untreated samples and after 6 h of fermentation. Differences in the NDF (22%) and hemicellulose (60%) contents of the treated and untreated samples could have resulted in higher digestibility values noted after 6 h of fermentation in the untreated samples. The implication was that proportions of the NDF and hemicellulose that would have been used by the fungi were available for use by the rumen microbes [64].

Table 2. Dry matter and fiber digestibility (% DM) of *Pleurotus ostreatus* treated and untreated corn stover (n = 8).

Time, h	Treatment	DMD [1], %	NDFD, %	ADFD, %	ADLD, %	HEMD, %	CELLD, %
6							
	Untreated	5.03 ^{f}	86.2 ^{a}	61.5 ^{c}	13.5 ^{d}	24.8 ^{a}	48.0 ^{cb}
	Treated	17.1 ^{e}	75.2 ^{d}	70.2 ^{a}	15.4 ^{cd}	5.02 ^{d}	54.7 ^{a}
24							
	Untreated	20.5 ^{d}	82.9 ^{b}	62.1 ^{c}	14.6 ^{d}	20.8 ^{b}	47.4 ^{c}
	Treated	30.0 ^{c}	78.5 ^{c}	70.2 ^{a}	18.5 ^{b}	8.30 ^{c}	51.8 ^{b}
48							
	Untreated	37.3 ^{b}	82.5 ^{b}	60.9 ^{c}	17.8 ^{bc}	21.7 ^{b}	43.1 ^{d}
	Treated	52.5 ^{a}	78.4 ^{c}	68.3 ^{b}	22.8 ^{a}	10.2 ^{c}	45.5 ^{cd}
SEM		1.832	0.45	0.49	0.51	0.884	0.61
p-values							
Time		<0.001	0.889	<0.001	<0.001	0.118	<0.001
Trt		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trt × Time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

$^{a–f}$ Means with different superscripts within each column differ by $p < 0.05$. [1] DMD, dry matter disappearance; NDFD, neutral detergent fiber disappearance; ADFD, acid detergent fiber disappearance; ADLD, acid detergent lignin disappearance; HEMD, hemicellulose disappearance; CELLD, cellulose disappearance; Trt, treatment.

3.4. Nutritive Value

It has been reported that the expression of ligninolytic enzymes potential of *P. ostreatus* is substrate dependent [67,68]. Results from the current study confirmed that the lignocellulose structure was broken which impacted the lignocellulosic complex and decreased ADF from 44.7 to 41.7% while NDF decreased from 71.3 to 58.3%, thereby, increasing NFC composition from 12.6 to 27.5% (Table 1). Additionally, the 58.5% increase in CP in the treated samples when compared with untreated samples, could provide more amino acid for tissue protein synthesis [30]. Consistent with the current study, numerous studies have also reported improvement in the chemical composition of *P. ostreatus* treated substrates [30,49,50,62,64,66,69]. The improvements in the nutritive value from the chemical composition data in the current study are in contrast to a previous study [18], which concluded that improving the nutritive value of corn stover as ruminant feed may not be a viable approach as none of the four white rot fungi (*Ceriporiopsis subvermispora, Lentinula edodes, P. eryngii,* or *P. ostreatus*) used in their study improved the nutritive value of corn stover. One could only speculate on the reason (possibly economic) why the authors reach that conclusion but the majority of studies in the literature reported an improvement in the nutritive value of treated substrates.

3.5. In Vitro Gas Production and Efficiency of Microbial Protein Synthesis

Significant interactions ($p < 0.001$) were detected for the total gas produced, partitioning factor, and microbial mass of *P. ostreatus* treated and untreated samples (Table 3). A difference of 681% in the microbial mass concentration of the lowest (untreated corn stover after 6 h of in vitro fermentation) and highest (treated corn stover at the same time) could be a result of higher CP which contributed additional amino acids and peptides to stimulate the growth rate and yield of ruminal microorganisms, resulting in an increase in microbial biomass synthesis. Consequently, the feed quality of the treated substrate is expected to improve as reported in a previous study which stated that microbial biomass synthesis has the potential to meet 70–100% of the protein needs of ruminants [70]. Higher microbial mass yield could also be related to higher fermentable carbohydrates from NFC in the treated (27.5%) versus the untreated (12.6%) samples. The difference which is 118% is significant and it has been documented that fermentable carbohydrates can increase microbial growth due to higher adenosine triphosphate (ATP) production per unit time

than per unit substrate [71]. Therefore, this is expected to increase microbial protein that will be available to the animal. This is consistent with the report by [72] who stated that modification and assimilation of nitrogenous compounds by fungi may improve the availability of protein in fungal-treated biomass for rumen microbes to utilize as microbial nitrogen. Hence there is great potential for high efficiency of microbial protein synthesis (EMPS), which provides most of the metabolizable protein supplied to the animal [61]. Additionally, Ref. [71] reported that amylolytic and propionate-producing bacteria could be more efficient with respect to microbial protein production due to their faster growth compared to cellulolytic bacteria. A significant ($p < 0.001$) decrease in partitioning factor (PF) in the treated samples after 48 h of in vitro batch culture fermentation could be a result of its higher gas production (187 mL) compared with the lowest gas volume (3.56 mL) noted for untreated samples at 6 h. Lower PF in the current study due to higher gas production is expected as total gas volume is one of the variables used in calculating PF. This is in contrast with [59,70] who reported higher values for both PF and microbial mass. The use of essential oil blends in [59] which is known to depress gas production could be responsible for the higher PF values. In [18], the authors reported that a decrease in gas production for *P. ostreatus* treated corn stover could be related to the proportion of lignin in the original substrate. Contrary to our earlier speculation of microbial mass yield and NFC, Ref. [73] reported no relationship between EMPS and NFC, although increasing fiber concentration was associated with a linear decrease in EMPS. Lower microbial mass noted for the untreated samples in the present study could be as a result of an imbalance in protein and carbohydrate concentrations. It is reported that amino acids and peptides can only stimulate microbial growth when the energy source permits a fast growth rate [71,73].

Table 3. In vitro gas production, dry matter digestibility, and microbial production efficiency of *Pleurotus ostreatus* treated and untreated corn stover ($n = 8$).

Time, h	Treatment	Gas, ml	PF [1]	Mm, g/kgDM	IVADDM	IVTDDM	Undegraded
6							
	Untreated	3.56 ^{f}	11.6 ^{a}	0.011 ^{e}	0.18 ^{e}	0.18 ^{f}	0.39 ^{a}
	Treated	13.6 ^{e}	10.1 ^{b}	0.087 ^{a}	0.20 ^{e}	0.38 ^{d}	0.32 ^{b}
24							
	Untreated	40.2 ^{d}	2.69 ^{c}	0.017 ^{de}	0.32 ^{d}	0.34 ^{e}	0.31 ^{b}
	Treated	63.3 ^{c}	2.37 ^{cd}	0.035 ^{c}	0.38 ^{c}	0.45 ^{c}	0.28 ^{c}
48							
	Untreated	113 ^{b}	1.52 ^{d}	0.031 ^{cd}	0.43 ^{b}	0.48 ^{b}	0.25 ^{d}
	Treated	187 ^{a}	1.39 ^{d}	0.064 ^{b}	0.50 ^{a}	0.63 ^{a}	0.19 ^{e}
SEM		5.76	0.502	0.0039	0.014	0.016	0.007
p-values							
Time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trt		<0.001	0.019	<0.001	<0.001	<0.001	<0.001
Trt × Time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1] PF, partitioning factor; Mm, microbial mass; IVADDM, in vitro apparent dry matter digestibility; IVTDDM, in vitro true dry matter digestibility; Trt, treatment. $^{a–f}$ Means with different superscripts within each column differ by $p < 0.05$.

3.6. In Vitro Dry Matter Digestibility

Significant interactions ($p < 0.001$) were detected for the IVADDM, IVTDDM, and undegraded residues of *P. ostreatus* treated and untreated corn stover. Consistent with the trend noted in DMD, the highest IVADDM and IVTDDM values were also noted in the treated samples after 48 h of in vitro batch culture fermentation. This increase in nutrient digestibility also resulted in a 105% decrease ($p < 0.001$) in the undegraded residues of the 48 h treated samples. Similarly, ref. [21], reported improvement in IVTDDM when corn straw was treated with *P. ostreatus*. The improvement in IVTDDM for treated corn stover

could be due to the breakup of the lignocellulosic structure by *P. ostreatus*. This will invariably increase animal intake and improve animal performance and production. Consistent with the present results, refs. [21,64] reported that biological treatment of roughages resulted in higher digestibility compared with untreated roughages. Additionally, refs. [69,74] reported that substrates degraded by mushrooms should be more digestible. Another previous study reported quicker and better digestibility in an in-situ study when *P. ostreatus*-treated maize straw was fed to sheep [56]. The improvement in DM in treated corn stover can be attributed to the ligninolytic enzymatic activity on the lignocellulosic complex. Contrary to the current results, ref. [18] reported that there was no improvement in the in vitro fermentability of maize stover treated with *P. ostreatus*. One can only speculate that the lower temperature (24 °C) used in that study could have affected the fermentation process resulting in lower fermentation of the stover.

It was observed that IVTDDM was not negatively impacted by the low NDFD and HEMD in the treated sample across the different incubation time points and this could have been impacted by higher NFC (27.5%) in the treated samples. Hence, it is speculated that feeding spent mushroom substrates alone might not provide enough effective fiber composition for ruminal efficiency, so it will be recommended that appropriate physical effective fiber is provided when feeding spent mushroom substrates. Finally, it can be concluded that higher NDFD in the untreated samples at all fermentation periods indicates the availability of a higher concentration of structural carbohydrates for ruminal microorganisms which tend to degrade starch first before shifting to degradation of fibrous carbohydrates [75].

3.7. Total Volatile Fatty Acid

Treatment x time interactions ($p < 0.05$) were detected for total volatile fatty acid (TVFA), acetate, propionate, butyrate, iso-butyrate, and acetate:propionate ratio (Table 4). Most of the variables were higher in the treated corn stover samples after 48 h of in vitro batch culture fermentation. Due to the higher molar proportion of propionate in the 48 h treated samples, the lowest acetate and acetate:propionate concentrations were noted for this treatment. Higher TVFA production in the 48 h treated samples would provide additional energy to animals as rumen fermentation is known to provide 70–85% of energy needs in ruminants [72]. Hence, this indicates that the decrease in hemicellulose and nutrient losses was compensated by improved digestibility across all three fermentation periods in the treated samples. The significant ($p < 0.001$) difference in NFC (27.5%) in treated samples might have favored amylolytic bacteria activity which resulted in a positive shift in the rumen fermentation pathway that significantly ($p < 0.001$) increased propionate production (4.92–7.83%). This is consistent with a previous report by [62], who stated that rapidly fermentable carbohydrates will result in an increase in propionate production. Thus, it implied that the solid-state fermented corn stover substrates were efficiently utilized despite the decrease in their OM, hemicellulose, and cellulose contents. The increase in acetate concentration in the untreated corn stover especially after 6 h of in vitro batch culture fermentation is consistent with the fermentation pathway for low-quality fibrous feed, as acetate production is increased when fermentable carbohydrate is limited [75].

Table 4. Total (mmol) and individual concentration of volatile fatty acid production of *Pleurotus ostreatus* treated and untreated corn stover (n = 8).

Time (h)	Trt	[1] TVFA	Acetate	Propionate	Butyrate	Iso-Butyrate (10^{-3})	Valerate (10^{-3})	Iso-valerate (10^{-3})	A:P Ratio
6									
	Untreated	29.5 ^{d}	0.739 ^{a}	0.183 ^{d}	0.069 ^{c}	2.03 ^{abc}	2.69	3.47	4.09 ^{a}
	Treated	33.3 ^{d}	0.726 ^{b}	0.192 ^{cd}	0.074 ^{c}	1.68 ^{c}	2.57	3.27	3.80 ^{b}
24									
	Untreated	42.7 ^{c}	0.719 ^{b}	0.201 ^{c}	0.071 ^{c}	1.85 ^{abc}	2.60	3.47	3.58 ^{b}
	Treated	46.7 ^{c}	0.694 ^{c}	0.216 ^{b}	0.082 ^{ab}	1.78 ^{bc}	2.84	3.77	3.22 ^{c}
48									
	Untreated	54.4 ^{b}	0.698 ^{c}	0.217 ^{b}	0.076 ^{bc}	2.14 ^{a}	3.01	4.13	3.24 ^{c}
	Treated	62.0 ^{a}	0.669 ^{d}	0.234 ^{a}	0.086 ^{a}	2.11 ^{ab}	3.42	5.03	2.87 ^{d}
SEM		1.49	0.0031	0.0025	0.0011	0.046	0.283	0.371	0.058
p-values									
Time		<0.001	<0.001	<0.001	0.002	0.014	0.714	0.433	<0.001
Trt		<0.001	<0.001	<0.001	<0.001	0.115	0.779	0.678	<0.001
Trt × Time		<0.001	<0.001	<0.001	<0.001	0.008	0.946	0.708	<0.001

[1] TVFA, total volatile fatty acids; Trt, treatment; A:P, acetate:propionate ratio. $^{a–d}$ Means with different superscripts within each column differ by $p < 0.05$.

3.8. Estimated Variables

The estimated variables were significantly ($p < 0.001$) impacted by the biological treatment of corn stover with *P. ostreatus* as shown in Table 5. The relative feed value for treated corn stover was significantly ($p < 0.001$) higher than untreated samples. Treatment also had a positive effect on the relative forage quality which could result in increased intake of the treated corn stover. Higher calculated dry matter digestibility, total digestible nutrient, and dry matter intake in the present study are consistent with this assumption. This improvement could have resulted from the reduction in the structural carbohydrates during the bioconversion process. Gross energy, digestible energy, and metabolizable energy were significantly higher ($p < 0.05$) in the treated samples vs. untreated. The implication is that better animal performance and productivity are expected from feeding the animals the treated corn stover. The net energy (net energy for maintenance, net energy for growth, and net energy for lactation) followed a similar trend as the other energy values. Digestible crude protein was significantly ($p < 0.001$) higher in treated corn stover compared with the untreated samples and this could have resulted from the improvement in CP content and available amino acids. Results from the estimated variables showed that bioconversion has the potential to improve the feeding value of corn stover to improve performance for increased productivity.

Table 5. Estimated dry matter intake, nutritive and energy values of *Pleurotus ostreatus* treated and untreated corn stover (n = 8).

Variable	Untreated	Treated	SEM	p-Values
DMI [1]	1.68 ^{b}	2.06 ^{a}	0.055	<0.001
TDN, %	46.8 ^{b}	50.7 ^{a}	0.75	0.006
DDM,	54.1 ^{b}	56.4 ^{a}	0.45	0.006
RFV	70.5 ^{b}	90.1 ^{a}	2.96	<0.001
RFQ	64.0 ^{b}	84.9 ^{a}	3.25	<0.001
DCP, g/kg$^{w0.75}$	0.04 ^{b}	1.88 ^{a}	0.276	<0.001

Table 5. *Cont.*

Variable	Untreated	Treated	SEM	*p*-Values
GE, MJ/kg DM	4.29 [b]	4.56 [a]	0.043	<0.001
DE, MJ/kg DM	2.06 [b]	2.23 [a]	0.033	0.006
ME, MJ/kg DM	1.69 [b]	1.84 [a]	0.027	0.006
NEM, MJ/kg DM	1.07 [b]	1.18 [a]	0.022	0.006
NEG, MJ/kg DM	0.35 [b]	0.46 [a]	0.022	0.006
NEL, MJ/kg DM	1.14 [b]	1.23 [a]	0.018	0.006

[1] DMI, dry matter intake, TDN, total digestible nutrient, DDM, digestible dry matter, RFV, relative forage value; DCP, digestible crude protein; GE, gross energy; DE, digestible energy; ME, metabolizable energy; NEM, net energy of maintenance; NEG, net energy of growth; NEL, net energy of lactation. [a,b] Means with different superscripts within each column differ by $p < 0.05$.

4. Conclusions

Previous and current results show that solid-state fermentation with white rot fungi could be a biological treatment alternative that may enhance the feeding quality of crop residues. The SSF of corn stover with *P. ostreatus* increased CP, ash, TVFA, propionate, microbial mass, IVADDM, IVTDDM, and DMD values, reduced some of the fiber fractions, and confirmed the potential for corn stover to be converted to animal feed with improved feed value enriched with fungal biomass. The impact of *P. ostreatus* on lignin was not significant probably due to the short period of SSF (3 weeks). This may imply that a longer time for SSF with *P. ostreatus* is necessary to achieve significant lignin degradation in corn stover. Therefore, there is a need to investigate the effect of different incubation periods on lignin degradation in corn stover.

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