

Evaluation of remnants of mushroom production (*Pleurotus ostratus* (Jacq. ex Fr.) P. Kumm) as an alternative for food in ruminants

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ABSTRACT

Objective: This study evaluated the residues from *Pleurotus ostreatus* mushroom cultivation as a potential alternative feed source for ruminants.

Design/Methodology/Approach: A completely randomized design was implemented with three treatments corn, oats, and sorghum each replicated four times. The physicochemical properties of the agricultural residues were analyzed both before and after mushroom cultivation. Parameters measured included dry matter (DM), crude protein (CP), ether extract (EE), ash (A), neutral detergent fiber (NDF), and *in vitro* digestibility. Statistical analyses were performed using the InfoStat software.

Results: Sorghum and oat residues exhibited higher carbon concentrations, whereas corn residues contained the greatest amount of NDF. Post-cultivation analysis revealed that oat residues presented an NDF content of 72.62%, exceeding that of corn and sorghum. Moreover, EE content increased in corn and sorghum residues, reaching 2.07% and 2.16%, respectively. A notable rise in CP was observed in oat residues, which reached 10.2%. In terms of *in vitro* digestibility, corn stover demonstrated the highest digestibility at 45 hours (65%), while oat stover exhibited the lowest.

Limitations/Implications: Identifying nutritionally rich and sustainable feed alternatives for ruminants remains critical to ensuring year-round availability and reducing dependency on conventional feed sources.

Findings/Conclusions: Residues derived from mushroom cultivation represent a promising nutritional resource for ruminant diets, particularly due to their enhanced protein and lipid profiles.

Keywords: Mushroom, Agro-industrial waste, Animal nutrition.

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INTRODUCTION

In Mexico, rising feed input costs comprising over 60% of total production expenses have become a significant challenge for livestock systems, necessitating the exploration of alternative feeding strategies to reduce these expenditures (Núñez-Torres, 2017). However, due to their unique digestive physiology, ruminants are capable of consuming low-quality agricultural by-products to meet their nutritional needs and convert them into products



suitable for human consumption (Rahman *et al.*, 2021). Despite this potential, agricultural industries often overlook the utilization of residues, which are typically discarded during processing. Nonetheless, these by-products possess nutritional value and can be repurposed for various environmentally and socioeconomically beneficial applications, such as compost production and mushroom cultivation (Vargas & Pérez, 2018). *Pleurotus ostreatus*, in particular, has the enzymatic capacity to degrade lignin in the substrates used for mushroom cultivation, contributing to nutrient transformation and the provision of trace elements (Leong *et al.*, 2022; Niego *et al.*, 2021). Therefore, the *in vitro* gas production technique represents a reliable alternative for assessing digestibility in animals, providing precise numerical data (Besharati *et al.*, 2020). Digestibility outcomes vary based on the composition and nutrient availability of the substrate, which determines the extent to which the feed can be metabolized and utilized by ruminants (Barnes & Marten, 1979). The aim of this study was to evaluate the nutritional characterization of *Pleurotus ostreatus* mushroom production residues as an alternative feed resource for livestock.

MATERIALS AND METHODS

Location of the experiment

The study was conducted at the Faculty of Agronomy and Veterinary Medicine, Autonomous University of San Luis Potosí (UASLP), Mexico, from September 12 to December 2, 2022. The UASLP is geographically located at 22° 09' N latitude and 100° 58' W longitude, at an altitude of 1,860 meters above sea level. The region has an average annual temperature of 26 °C and an average annual precipitation of 341 mm. The prevailing climate is classified as warm sub-humid (García, 1988).

Experimental method

Three treatments with four replicates each were used: corn, oat, and sorghum residues derived from the spent substrate of *Pleurotus ostreatus* mushroom production. These substrates were collected from the mushroom cultivation module, corresponding to the November 2021 production cycle.

Chemical analysis

At the onset of the mushroom cultivation project, individual compositional analyses were performed on the three agricultural residues, following the procedures established by AOAC (1990). The parameters assessed included dry matter (DM), crude protein (CP), ether extract (EE), ash (A), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Upon completion of mushroom cultivation, a comparative analysis was conducted on the residual substrates, evaluating the same components (DM, CP, EE, A, NDF, and ADF) using the same AOAC (1990) methodology.

In vitro dry matter digestibility

Rumen fluid was collected from 1-year-old Rambouillet sheep housed in individual 1.5×1 m pens equipped with feeders and waterers. A total of 40 mL of fasting rumen fluid was extracted from each donor animal using a connected rumen probe. The rumen

fluid was immediately transported to the UASLP bromatology laboratory in sterile 150 mL thermal containers at 36 °C to preserve the viability of the ruminal microorganisms. Subsequently, 100 g of each treatment (sorghum straw, oat straw, and corn stover) were dried at 65 °C for 24 hours. The dried samples were then ground and sieved through a 1 mm mesh and placed in 100 mL amber bottles. The rumen fluid was filtered through four layers of cheesecloth into a flask and mixed with a buffer solution (Table 1). Each treatment was combined with the buffered rumen fluid and incubated in 100 mL amber bottles in a water bath (Daihan equipment) at 40 °C under constant agitation for 72 hours. After incubation, the samples were removed, washed with hot water, and filtered. Residual dry matter content was determined using a drying oven, and dry matter digestibility was calculated for each treatment using the formula proposed by Ørskov *et al.* (1980).

Estimation of *in vitro* gas production

The production of CO₂ and CH₄ gases was estimated using the *in vitro* gas production technique over a 72-hour incubation period, as described by Theodorou *et al.* (1994). For each treatment, 0.5 g of substrate was placed in 100 mL amber bottles, to which 90 mL of rumen fluid was added. The bottles were sealed with rubber stoppers and incubated in a water bath at 39 °C. At the end of the incubation period, the volume of gas produced from the fermentation process was measured.

Statistical analysis

A completely randomized design was employed with three treatments corn, oats, and sorghum residues from mushroom substrate each replicated four times. The measured variables were subjected to analysis of variance (ANOVA), and treatment means were compared using Tukey's test at a significance level of $p \leq 0.05$. All statistical analyses were performed using the InfoStat software (Di Rienzo, 2020).

RESULTS AND DISCUSSION

Proximate analysis

The stover samples exhibited a similar nutrient profile across treatments. However, sorghum and oat residues showed higher ash content, which may be attributed to soil contamination during the baling process, as this stage often involves the collection of ground

Table 1. Reagents used in the buffer solution.

Reagent	Quantity (g L ⁻¹)
KH ₂ PO ₄ (anhydrous potassium phosphate)	1.0
MgSO ₄ ·7H ₂ O (magnesium sulfate heptahydrate)	0.5
NaCl (sodium chloride)	0.5
CaCl ₂ ·2H ₂ O (calcium chloride dihydrate)	12.0
CH ₄ N ₂ O (urea)	0.5
Na ₂ CO ₃ (sodium bicarbonate)	15.0
Na ₂ S·9H ₂ O (sodium sulfide nonahydrate)	1.0

Tilley y Terry, 1963.

particles. Corn stover demonstrated the highest level of neutral detergent fiber (NDF), which corresponds to the detergent-insoluble fraction comprising cellulose, hemicellulose, lignin, and silica components that form the plant cell wall. The remaining variables showed minimal numerical variation among treatments (Table 2).

The substrates obtained after the inoculation process for *Pleurotus ostreatus* mushroom cultivation showed a reduction in acid detergent fiber (ADF) content in the residual materials. These results are consistent with findings by Ribeiro *et al.* (2018), who observed similar outcomes when using barley straw. This reduction is likely due to the ability of *Pleurotus* to degrade indigestible components such as lignin (Bermúdez-Savón *et al.*, 2023). Neutral detergent fiber (NDF) content decreased by approximately 5%, a reduction considered acceptable for ruminant feed. These findings differ from those reported by Zheng *et al.* (2021), who noted a 1% increase in fat content in oat substrates after *Pleurotus ostreatus* cultivation. In the present study, fat content increased numerically in corn and sorghum stovers compared to oats. Nevertheless, the overall results align with those of Palangi *et al.* (2022), who investigated various substrates for mushroom production. Post-inoculation, crude protein content was highest in oat residues, reaching 10.2%. Similar increases in crude protein were reported by other researchers using wheat straw (Fazaeli & Masoodi, 2006; Palangi *et al.*, 2022). This enhancement is likely due to enzymatic activity by the mushrooms for fiber degradation, as well as residual mycelium that may remain attached to the substrate (Table 3).

***In vitro* digestibility**

The results of dry matter digestibility (DMD) indicated higher digestibility percentages at 45 hours in the agricultural residues and across the treatments derived from the spent mushroom substrates. Compared to previous studies, these results reflect improved digestibility, as Shrivastava *et al.* (2012) reported a lower digestibility of 33.39% when using wheat straw (Table 4).

Dry matter digestibility of the stover varied depending on the incubation time when used as a substrate for *Pleurotus ostreatus* cultivation. It was observed that oat and corn stovers exhibited the highest digestibility at 45 hours, compared to the other agricultural residues used in mushroom production (Figure 1).

Table 2. Chemical Composition of Dried Agricultural Residues from Corn, Sorghum, and Oat Stover.

Analysis (%)	Dry agricultural waste		
	corn	oats	sorghum
Dry matter	94.16 ^b	93.7 ^a	94.4 ^b
Ash	6.70 ^a	7.61 ^b	10.13 ^c
Neutral detergent fiber	79.12 ^c	73.10 ^b	72.30 ^a
Acid detergent fiber	51.1 ^a	50.91 ^b	40.62 ^b
Ether extract	0.92 ^a	1.15 ^b	1.40 ^b
Crude protein	5.84 ^b	5.18 ^b	4.43 ^a

Measurements with different letters in the same row are significantly different ($p \leq 0.05$).

Table 3. Chemical Composition of Residual Substrates from *Pleurotus ostreatus* Cultivation Using Dried Agricultural Stover from Corn and Sorghum.

Analysis (%)	Treatments		
	corn	oats	sorghum
Dry matter	23.7 ^a	23.4 ^a	23.5 ^a
Ash	7.33 ^a	9.19 ^b	9.88 ^b
Neutral detergent fiber	66.38 ^a	72.62 ^c	69.42 ^b
Acid detergent fiber	39.75 ^a	39.34 ^a	37.69 ^a
Ether extract	2.07 ^b	1.45 ^a	2.16 ^b
Crude protein	6.08 ^b	10.2 ^c	5.00 ^a

Values followed by different letters within the same row are significantly different ($p \leq 0.05$).

Table 4. Time and Percentage of Dry Matter Digestibility in Agricultural Residues and Mushroom Production Substrates of *Pleurotus ostreatus*.

Agricultural Residues	Hours				
	10	20	30	40	45
Oats	58 ^a	61 ^a	63 ^a	65 ^a	67 ^a
Corn	63 ^c	68 ^c	71 ^c	71 ^b	72 ^b
Sorghum	59 ^b	66 ^b	69 ^b	70 ^b	70 ^b
Remnants of agricultural residues	Hours				
	10	20	30	40	45
Oats	56 ^b	61 ^c	63 ^c	64 ^c	65 ^b
Corn	51 ^b	53 ^b	54 ^b	56 ^b	65 ^b
Sorghum	44 ^a	46 ^a	47 ^a	49 ^a	50 ^a

Values followed by different letters within the same row are significantly different ($p \leq 0.05$).

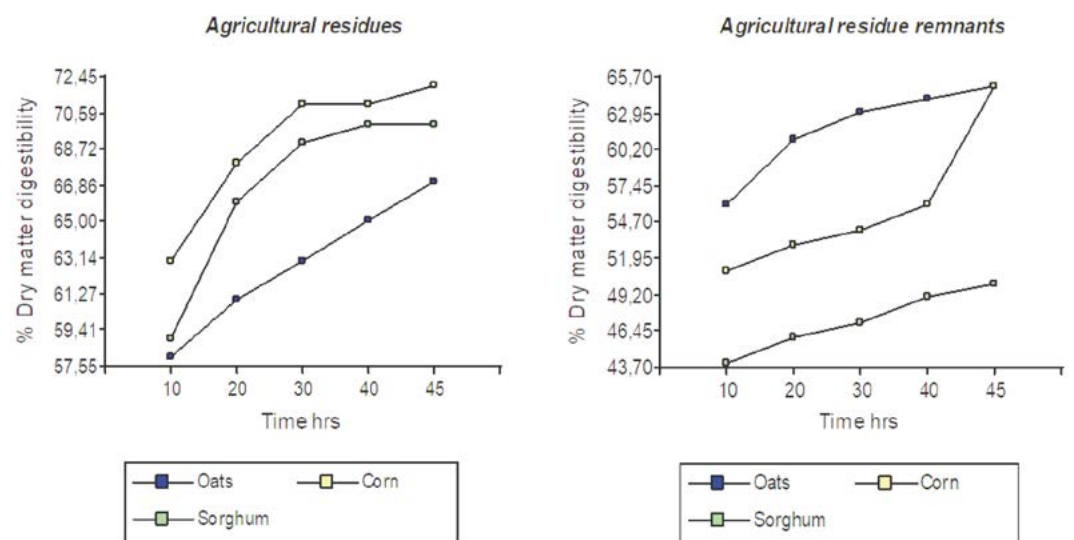


Figure 1. *In vitro* rumen digestibility of stover before and After Inoculation Used in *Pleurotus ostreatus* Mushroom Cultivation.

In vitro dry matter digestibility was highest in corn and oat stovers, both before and after their use as substrates for mushroom cultivation. In contrast, sorghum stover exhibited the lowest digestibility. These findings are consistent with those reported by Akinfemi (2010), who observed 60.90% digestibility in agricultural residues.

CONCLUSIONS

All three types of stover are suitable for use as substrates in *Pleurotus ostreatus* cultivation. Additionally, the resulting spent substrate demonstrates improved nutritional properties specifically in fat and protein content making it a viable alternative feed source for livestock.

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