Protein Fraction, Mineral Profile, and Chemical Compositions of Various Fiber-based Substrates Degraded by *Pleurotus ostreatus*

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The objective of this study was to characterize the substrates after their degradation by *P. ostreatus* within 60 days of cultivation in four Mexican mushroom-producing companies, in order to use it as a complement feed for ruminants. The acid detergent fiber (ADF), neutral detergent fiber (NDF), protein fraction, and mineral profile were analyzed after degradation. Crude protein (CP) of companies 1 and 4 increased by 6.1% and 6.8%, respectively ($p \leq 0.05$). Soluble nitrogen reached 60% relative to un-degraded substrate with 43.8%. Proteins A, B1, and B3 fractions increased compared to controls ($p \leq 0.05$) at some companies. The B2 fraction was decreased in all the degraded substrates ($p \leq 0.05$), but the C fraction at companies 1, 2, and 3 presented no significant differences with respect to their controls ($p \leq 0.05$). Crude fiber (CF), ADF, NDF, and hemicellulose (HC) decreased while dry matter digestibility (DMD) increased to 55.5-58% on degrade substrates. The mineral composition increased disproportionately. The substrate degraded by *P. ostreatus* by improving its digestibility and soluble protein content may be a low-cost food supplement. However, due to its mineral imbalance, it is not recommended as the sole food source for ruminants.

Keywords: Protein fractions; Bromatological profiles; Microelements; Residues degraded; Fungi; Animal feed

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INTRODUCTION

Agricultural residues such as straw and husks have been used as feed for ruminants despite their high fiber content. However, the strong bonds between cellulose, hemicellulose, and lignin impede the microorganisms in the rumen from fully utilizing the carbohydrate content (cellulose and hemicellulose) (Van Kuijk *et al.* 2015). In principle, enhancing the digestion of these food sources can be achieved by delignifying the fiber content by physical and/or chemical means. Nonetheless, this involves two disadvantages, as it increases costs, and affects the environment. Biological processes using fungi such as *Pleurotus ostreatus*, in contrast, offer benefits: the production of fructiferous bodies apt for human consumption and, simultaneously, degraded materials with high enzyme and metabolite contents. The changes that substrates undergo after fungal growth, are due to the excretion of enzymes, radicals, and other chemical compounds that degrade the lignocellulose into polysaccharides.

As the fructiferous body develops, the nitrogen in the lignocellulosic biomass is incorporated into the fungal protein to increase the nitrogen concentration (crude protein) and improve the nutritional quality of vegetable matter (Van Kuijk *et al.* 2015).

Mushroom production in the world has increased by 30% since 1978, exceeding 4.70 kg per capita annual consumption (Royse *et al.* 2017). China is the leading producer of edible mushrooms; on the other hand, Mexico is positioned as the largest producer in Latin America, generating around 80.8% of the total production in the region (Romero-Arenas *et al.* 2015; Bellettini *et al.* 2019). In 2011, the production of fresh mushrooms in México reached 62,374 t, increasing every year, of which 4.76% corresponds to mushrooms (*Pleurotus* spp.), (Martínez-Carrera *et al.* 2016). The State of Hidalgo is Mexico's third-largest producer. Even though mushroom-growing is an important economic activity there, it is also a source of pollution, since the substrate generated by cultivation accumulates because it has no specific use. The modified composition of these substrates has drawn attention; however, they have been proposed as a food source for ruminants (Van Kuijk *et al.* 2015). Most research on biologically-degraded materials have focused on the fungus' effect on fiber (ADF, NDF, lignin) and digestibility. Few studies have set out to determine the composition of the nitrogenized compounds or the mineral profile of biologically-delignified material, despite their importance for animal health and formulating animal diets. Adequate supplies of mineral elements in feed supports the appropriate functioning of animal organisms, metabolic processes, and interactions between them, but inadequate administration in diets may result in deficiencies or excesses that can cause disease or even death (Calsamiglia *et al.* 2009; Spears and Weiss 2014).

The Cornell Net Carbohydrate and Protein System (CNCPS) has a sub-model that predicts rates of feedstuff degradation in the rumen, the passage of undegraded feed to the lower gut, and the amount of energy and protein that is available to the animal. In the CNCPS, structural carbohydrate (SC) and nonstructural carbohydrate (NSC) are estimated from sequential neutral detergent fiber analyses of the feed. Data from literature are used to predict fractional rates of SC and NSC degradation. Crude protein (CP) is partitioned into five fractions as a function of solubility in precipitating agents, buffer solutions, and detergent solutions (Sniffen *et al.* 1992). Fraction A represents the soluble protein considered as non-protein nitrogen (NNP), which is assumed to undergo degradation quickly into ammoniacal nitrogen in the rumen and, together with carbon skeletons and energy in the form of ATP, participates in the synthesis of microbial protein. Fraction B1 is a true soluble protein; B2 is a protein with intermediate degradation rates that partially degrades in the rumen and small intestine; and B3 is considered an escape or bypass protein since it degrades very slowly in the rumen due to its bonding to the cell wall so it can go through the small intestine, where it is used by the animal. Finally, fraction C is an unavailable protein because it bonds to lignin (Lanzas *et al.* 2008; Van Soest 2018; Nayan *et al.* 2018).

In light of the foregoing, the high generation of residues degraded by *P. ostreatus*, and the biological advantages they offer, make them ideal candidates for use as animal feed; however, characterizations of the protein fractions and mineral content of these residues are scarce. Therefore, the main objective of this study was to characterize the various fiber substrates degraded by *P. ostreatus*, for 60 days of cultivation, at four mushroom-producing companies in Hidalgo, Mexico, in order to assess their possible use as a supplement in ruminant feedings.

EXPERIMENTAL

Obtaining the Vegetable Matter

A sample of the fiber-based substrate was obtained prior to cultivation as a control sample. Six samples of degraded substrates were taken after 60 days of cultivation with *P. ostreatus* in various locations of Tolcayuca (19º 57´ 24´´ N, 98º 55´ 13´´ W) (Company 1), Villas de Tezontepec (19º 52´ 47´´ N, 98º 49´ 09´´ W) (Company 2), Progreso de Obregón (20º 14´ 53´´ N, 99º 11´ 23´´ W) (Company 3), and Cuautepec de Hinojosa (20º 09´ 00´´ N, 98º 26´ 00´´ W) (Company 4), in Hidalgo. The companies used substrates that contain barley straw, corn chaff-barley straw (50:50), corn chaff-oat straw (50:50), and corn chaff, respectively. The companies, as listed, use different strains of *P. ostreatus*: (Company 1: BPR-3 strain; Company 2: PR5 strain; Company 3: L85 C11 strain; Company 4: *Pleutorus* spp. Strain).

Physicochemical Characterization

Bromatological analyses were performed following the methodologies proposed by the AOAC (AOAC 2005) to determine moisture (M) (925.10), crude protein (CP) (960.52), ethereal extract (EE) (945.16), crude fiber (CF) (962.09), and ash (923.03). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined using the methodology of Van Soest *et al.* (1991). Hemicellulose (HC) was calculated from the difference between NDF and ADF. Protein fractions (A, B1, B2, B3, C) were performed following the method of Krishnamoorthy *et al.* (1982), while mineral composition was obtained from atomic absorption spectrometry (AOAC 2005).

Dry Matter Digestibility

The digestibility percentage of the dry matter (% DMD) was calculated using Eq.1 (Linn and Martin 1991; Boga *et al.* 2014).

$$DMD\ (\%) = 88.9 - 0.779*(ADF) \quad (1)$$

Statistical Analyses

The data obtained in triplicate had a completely random arrangement. Each independent variable was subjected to a normality analysis using the Shapiro-Wilk test in the PAST computer program, version 3.21 (Hammer 2018), and a variance analysis (ANOVA) with $P < 0.05$. To examine the differences between controls and the degraded substrates for all the analyzed parameters, a Student-t test was performed with $P < 0.05$. The comparison between the means of the companies was analyzed by applying a Tukey test for multiple ranges with $P < 0.05$, using Minitab 18.

RESULTS AND DISCUSSION

The industry dedicated to the production of edible mushrooms generates as a by-product a high amount of degraded substrate worldwide, which is little used and is often discarded, burned or accumulated, generating serious pollution problems. During colonization and growth, fungi improve the nutritional value of the residues, increasing the content of vitamins, protein, and digestibility (Villas-Bôas *et al.* 2002); however, in order

for these substrates to be incorporated into the ruminant diet, it must be ensured that they have an increase in crude protein and digestibility.

There are several studies on the chemical characterization of post-cultivation degraded substrates of fungi, which show that the strain, type of substrate used, cultivation technology, and geographical area, are some of the factors that influence the nutritional quality and digestibility of degraded substrates (Ruiloba *et al.* 2014). Variations due to these factors have limited their use. A lack of a standardized production process, as wells as the handling of different substrates and strains, gives rise to differences in chemical composition in degraded substrates. Therefore, it is necessary to carry out research for each region and in this way to establish whether or not they are candidates as supplements for ruminants.

In this sense, this research found that the comparative analysis of the chemical composition of the residue without degrading and those obtained after 60 days of cultivation, showed statistically significant changes ($P \leq 0.05$) in material from all the companies under study, with an increase in CP, EE, ash, DMD, soluble protein and decrease in CF, ADF, NDF, HC, and insoluble protein (Table 1 and 2). Proteins A, B1, and B3 fractions increased compared to controls ($p \leq 0.05$) at some companies (Table 3 and 4). Company 4 presented the best bromatological analysis results, followed by companies 3, 2, and 1. These improvements can be attributed to the type of strain used, which was the most efficient in degrading different fiber fractions that are present in corn straw, reflecting in a significant decrease in NDF that corresponds to the fibers of the cell wall (lignin, cellulose and hemicellulose), ADF, and HC. The content of ethereal extract increased in all degraded residues relative to its controls. There have been no reports that explain this behavior, possibly due to the production of fatty acids during the growth of the mycelium and fruiting bodies of the fungus.

These results confirm that when using substrates rich in lignin such as oat and barley straw, biodegradation is limited and the strain plays an important role in the ability to produce enzymes that allow it to degrade lignin. This makes it possible to access the sugars present in hemicellulose and cellulose in order to take advantage of them as energy sources during the colonization of the substrate and its subsequent fruiting (Anike *et al.* 2016). *Pleurotus ostreatus* is a white rot fungus identified as a species capable of improving the nutritional conditions of lignocellulosic materials because of its high selectivity for degrading lignin (Van Kuijk *et al.* 2015).

On the other hand, the proportion of ash in the degraded substrates in comparison with their controls increased significantly ($P \leq 0.05$) (Tables 1 and 2). It has been mentioned that this increase is due to the addition of calcium sulfate and calcium carbonate to the substrates during the cultivation of the fungi; therefore, increases the ash content in the degraded residues, generating an imbalance in the minerals and limiting its use as ruminant feed (Urrego *et al*. 2013; Pineda-Insuasti *et al.* 2014; Zhang *et al.* 2014). Adamović *et al.* (1998) found higher ash content in substrate degraded by *P. ostreatus* over wheat straw after 30 days. Ruiloba *et al.* (2014), who reported similar behavior for ash in substrate of rice straw degraded by strains RN82 and RN81 of *Pleurotus djamor*. Increased ash concentration is frequently reported on fungal degraded substrates and indicates the presence of organic matter degradation (Assi and King 2008; Koutrotsios *et al.* 2014; Atila 2017, Nasehi *et al.* 2017; Wanzenböck *et al.* 2017).

For mineral composition analysis, the degraded substrates from company 1, which had the lowest ash content was selected. The percentages of the macro-minerals P, Ca, Mg, Na, and K were 0.1%, 17.91%, 0.31%, 0.14%, and 1.67%, respectively, while those of the

micro-minerals Cu, Zn, and Fe were 35.71, 40.60 and 40.72 ppm, respectively. Compared with De Blas *et al.* (2010), the P, Ca, Mg, Na, K, Cu, and Zn contents increased; unlike Fe, that decreased in concentration. Murillo and Suárez (2020) identified the increase in P, Mn, Fe, Zn, and Cu of the substrate degraded by *Lentinus crinitus* and decreased the contents of ash, Ca, and Mg.

The increase observed for Ca, Mg, P, and K in the degraded substrate after 60 days might be due to the addition of mineral supplements commonly applied to *Pleurotus* spp. to improve yield. One additive is $CaCO_3$, which is applied to control pH, maintain moisture, prevent compaction of the substrate, and foster development of hyphae and fructiferous bodies (Pineda-Insuasti *et al.* 2014). Other supplements, including lime, ground limestone, KH_2PO_4, $MgSO_4.7H_2O$, and $Ca(NO_3)_2.4H_2O$ that all are rich in nitrogen, phosphates, and magnesium are useful for the growing of strains and degrading lignocellulose. In some cases, adding Cu or Mn enhances the peroxidase production and thereby optimizes substrate degradation. However, adding mineral supplements is a measure that only considers the economic efficiency of processes, but does not take into account the effects on the degraded substrate for its final application as ruminant feed (Levin *et al.* 2008; Sharma and Arora 2010).

Akinfemi and Ogunwole (2012) found that rice husk inoculated with *P. ostreatus*, *P. pulmonaris*, and *P. tuber-reguim* presents a disproportionate Ca:P ratio similar to that which was observed in this study. This imbalance can cause alterations in the ruminants organisms; for example, when the ratio is below optimum (Ca:P ratio of 1:1 to 2:1), dairy cattle tend to suffer hypocalcemia, while male sheep and goats may develop obstructive urolithiasis (urinary stones). In contrast, diets lacking Ca or P may result in abnormal bone development, a condition known as rickets in young animals and osteomalacia in adults (NCR 2007). Another mineral that limits the utility of these substrates, as the only food source for sheep is Cu, since concentrations in raw matter above 15 to 20 ppm, accompanied by low Mo levels, can cause intoxication and death (Calsamiglia *et al.* 2009).

In other results, the percentage of CF decreased significantly ($P \leq 0.05$) in all the degraded substrates, compared to controls (Tables 1 and 2). This reduction was because of the use of carbohydrates including cellulose, and the lignin polymer during fungal growth (mass transfer) and metabolism, where they are utilized as an energy source (Shrivastava *et al.* 2014; Nayan *et al.* 2018). The ADF, NDF, and HC contents (Tables 1 and 2) decreased with respect to their controls ($P \leq 0.05$). A reduction of the ADF and NDF has been reported in fungal-treated substrates (Lynch *et al.* 2014; Nasehi *et al.* 2014; Shrivastava *et al.* 2014; Nasehi *et al.* 2017) as a consequence degradation of the cell-wall component of the substrates by extra cellular enzymes of fungus (Sánchez 2009; Nasehi *et al.* 2017).

The use of cell wall fibers by white rot fungi depends on their characteristics as well as on the regulation mechanisms of lignin cellulolytic enzymes, where high concentrations of simple carbohydrates repress the expression of enzymes (Rouches *et al.* 2016). Therefore, the different species of *Pleurotus* that are used in the production processes of edible fungi can use the components of the cell wall differently depending on the type of substrate, cultivation time, interaction of the mycelium with the fibers due to the expression of extracellular enzymes, as well as other biotic and abiotic factors.

Adamović *et al.* (1998) reported reductions of NDF and ADF in wheat straw fermented with *P. ostreatus*, and a similar tendency for HC that are similar to those observed in the substrates analyzed herein. Luna *et al.* (2013) reported the same behavior in barley chaff inoculated with the IE8 strain of *P. ostreatus*. The NDF is related to dry

matter consumption, ruminal fermentation processes, and the generation and composition of the milk that produced by animals (Calsamiglia *et al.* 2009) and the ADF content is an indicator of the lignin which is present in plants and lignocellulosic residues, among other materials. Lignin is the main determinant of rumen digestibility. Thus, there is a relation between the amount of lignin present and the feed's digestibility, so that as ADF concentrations increased, the ingestion and digestibility of the dry matter (%DMD) decreased (Espinoza-Canales *et al.* 2017; Nayan *et al.* 2018).

The %DMD of the substrates degraded by *P. ostreatus* in this study increased from 54 to 57% ($P \leq 0.05$). Although ADF and NDF values decreased in all degraded substrates analyzed in this study, no significant changes in %DMD were observed in the different companies. This may be due to the percentages of lignin present as well as the fiber content in them. That is why even though company 4 goes from 48.5% to 42.4% of ADF, its %DMD is 55.9%, which is similar to company 3 and lower than company 1 and 2. Possibly these percentages of ADF and NDF help in fostering the utilization of carbohydrates and their use by microorganisms in the rumen to produce the microbial protein that participates in the formation of energy for product maintenance and synthesis (Rodríguez and Rodríguez 2007). Luna *et al.* (2013) observed an increase greater than 42% in the digestibility of the substrate of barley chaff degraded by *P. ostreatus* IE8 after 30 days of cultivation when utilizing the *in vitro* digestibility technique.

The CP content differed in all analyzed substrates. Those obtained from companies 1 and 4 increased by of 6.1% and 6.8%, respectively ($P \leq 0.05$), unlike the undegraded substrate. These values correspond to those reported with fibrous materials without degrading, for which the CP content is 4.6% (Urrego *et al.* 2013). The increase in protein is evident, and this can be attributed to the protein contained in the cereal grains used for the preparation of the primary inoculum used by each company, the fungal biomass generated after the degradation of carbohydrates, exogenous enzymes, stem residues, and fructiferous bodies that remained after harvesting (Wang *et al.* 2001; Nayan *et al.* 2018). The increase in soluble protein of all the analyzed substrates was confirmed, as also reported by Nayan *et al.* (2018).

It seems that *P. ostreatus* decomposed nonprotein nitrogen from substrate lignoprotein and synthesized the released nitrogen into fungal protein (Assi and King 2008). Of the total CP in all the degraded substrates, 60% represented soluble nitrogen in phosphate borate buffer, in contrast to the undegraded substrate, which measured 43.8% (Tables 1 and 2).

The protein that is soluble in phosphate-borate is known as a soluble protein, and it is assumed to degrade quickly in the rumen. This fraction is primarily composed primarily of non-protein compounds, such as ammoniacal nitrogen, urea, nitrates, amino-acids, small peptides, and true protein (Mohamed and Chaudhry 2008; Mahesh *et al.* 2017). Urrego *et al.* (2013) evaluated the composition of the nitrogenated compound of the residue of a crop of *Agaricus bisporus* from a Colombian company that used rice husks, sugarcane bagasse, poultry litter, and cotton as culture bed. Those authors observed that 76% of the total nitrogen content in borate-phosphate buffer consisted of mainly insoluble nitrogen. Regarding the fractions of CP, based on their degradation in the rumen, fraction A presented an increase compared to controls ($P \leq 0.05$). The substrate from company 2 showed the largest change of 9% to 34%, while the others remained between 28% and 37% (Tables 3 and 4).

Table 1. Nutritional Composition of the Substrates (controls) Used in the Cultivation of *P. ostreatus* by Four Companies in Hidalgo

Determination	Company 1	Company 2	Company 3	Company 4
Moisture (M) (%)	8.6 ± 0.2	8.1 ± 0.8	7.6 ± 0.6	8.9 ± 0.2
Crude protein (CP) (%)	4.9 ± 0.2	4.4 ± 0	4.8 ± 0	4.4 ± 0
Crude fiber (CF) (%)	56.5 ± 0.5	49.6 ± 0.3	48.4 ± 0.3	49.5 ± 0.3
Ethereal extract (EE) (%)	5.7 ± 0.4	8.3 ± 0.5	2.7 ± 0	7.8 ± 0.9
Ash (%)	7.2 ± 0.1	10.7 ± 0.7	11.5 ± 0.4	14.9 ± 0.7
ADF (%)	42.2 ± 0.7	43.1 ± 0.5	44.9 ± 0.2	48.5 ± 0.8
NDF (%)	75.7 ± 0.1	73.4 ± 0.9	75.4 ± 0.3	74.7 ± 0.1
HC (%)	33.5 ± 0.6	30.4 ± 0.4	30.5 ± 0.1	26.1 ± 0.7
DMD (%)	56 ± 0.5	55.4 ± 0.3	54 ± 0.1	51.1 ± 0.6
Soluble protein	2.3 ± 0	1.3 ± 0	2.3 ± 0.2	2.2 ± 0
Insoluble protein	2.6 ± 0	3.1 ± 0	2.5 ± 0.2	2.2 ± 0

Company 1: Substrate of barley chaff/strain *P. ostreatus* BPR-3 (Tolcayuca); Company 2: Substrate corn chaff – barley straw (50:50)/strain *P. ostreatus* PR5 (Villas de Tezontepec); Company 3: Substrate corn chaff – oat straw (50:50)/strain *P. ostreatus* L85 C11 (Progreso de Obregón); Company 4: Substrate corn chaff/strain *Pleutorus* spp. (Cuautepec de Hinojosa). ± is for SD.

Table 2. Nutritional Composition of Substrates Degraded by *P. ostreatus* after 60 Days of Cultivation from Four Companies in Hidalgo

Determination	Company 1	Company 2	Company 3	Company 4
Moisture (M) (%)	7.7 ± 0.7^{a}	6.8 ± 0.2^{b}	6.5 ± 0.2^{bc}	6.1 ± 0.3^{c}
Crude protein (CP) (%)	6.1 ± 0.9^{a}	4.4 ± 0.3^{b}	4.9 ± 0.2^{b}	6.8 ± 0.3^{c}
Crude fiber (CF) (%)	54.1 ± 0.7^{a}	45.5 ± 0.3^{b}	45.0 ± 0.5^{b}	42.7 ± 2^{c}
Ethereal extract (EE) (%)	6.2 ± 0.3^{a}	8.0 ± 0.5^{b}	7.9 ± 0.9^{b}	11.0 ± 0.9^{c}
Ash (%)	8.3 ± 0.1^{a}	20.2 ± 0.5^{b}	31.6 ± 0.7^{c}	33.1 ± 0.7^{d}
ADF (%)	40.8 ± 0.3^{a}	39.7 ± 0.6^{b}	42.9 ± 0.4^{c}	42.4 ± 0.7^{c}
NDF (%)	65.8 ± 2^{a}	68.0 ± 0.9^{b}	58.6 ± 0.6^{c}	51.6 ± 0.5^{d}
HC (%)	25.1 ± 2^{a}	28.3 ± 0.7^{b}	16.2 ± 0.7^{c}	9.2 ± 0.8^{d}
DMD (%)	57.1 ± 0.2^{a}	58 ± 0.4^{b}	55.5 ± 0.3^{c}	55.9 ± 0.5^{c}
Soluble protein	3.9 ± 0.1^{a}	2.6 ± 0.1^{b}	2.7 ± 0.1^{b}	3.1 ± 0.2^{c}
Insoluble protein	2.1 ± 0.1^{a}	1.8 ± 0.1^{b}	2.2 ± 0.1^{b}	3.7 ± 0.2^{c}

Company 1: Substrate of barley chaff/strain *P. ostreatus* BPR-3 (Tolcayuca); Company 2: Substrate corn chaff – barley straw (50:50)/strain *P. ostreatus* PR5 (Villas de Tezontepec); Company 3: Substrate corn chaff – oat straw (50:50)/strain *P. ostreatus* L85 C11 (Progreso de Obregón); Company 4: Substrate corn chaff/strain *Pleutorus* spp. (Cuautepec de Hinojosa). The a, b, c, and d between columns indicate significant differences ($P < 0.05$); and ± is for SD.

This fraction corresponds to the ammoniacal nitrogen, which is available to generate microbial protein (Nayan *et al.* 2018). Regarding fraction B1 (soluble nitrogen), the substrate from company 1 increased from 18% to 29% ($P \leq 0.05$), but fraction B2 decreased in all substrates (Tables 3 and 4) ($P \leq 0.05$). Fraction B3, the bypass protein, increased significantly in the degraded substrates from companies 2 and 4. Fraction C, the lignin-linked protein in the degraded substrates from companies 1, 2, and 3 showed no significant differences ($P \leq 0.05$). WingChing-Jones and Retana (2009) analyzed the nitrogenated lignin-linked compound from transvala hay that is used as a substrate in *P.*

ostreatus production. They found that 49.3% of total CP corresponded to fraction C; a higher percentage than the one determined in this study, which averaged 27%. They concluded that transvala hay inoculated with *P. ostreatus*, alone, is not a suitable source of protein for animal feed. Van Soest (2018) suggests that the range of lignin-linked CP in feed should be 3% to 5%, but due to their physical characteristics, the transvala hay, bales, and chaff inoculated with *P. ostreatus* can keep the ruminal system healthy since they stimulate rumination as long as the amount administered is adequate for the species.

Table 3. Protein Fractions of Various Substrates Used (controls) in the Cultivation of *P. ostreatus* at Four Companies in Hidalgo

Company	Fraction									
	A	(%)	B1	(%)	B2	(%)	B3	(%)	C	(%)
1	1.5 ± 0	30	0.9 ± 0	18	0.9 ± 0	18	0.3 ± 0	7	1.3 ± 0.4	27
2	0.4 ± 0	9	0.9 ± 0	20	1.3 ± 0	30	0.4 ± 0	9	1.4 ± 0.4	32
3	1.2 ± 0.4	25	1.1 ± 0.4	23	0.8 ± 0	17	0.4 ± 0	8	1.3 ± 0.4	27
4	1.3 ± 0	30	0.9 ± 0	20	0.5 ± 0	12	0.8 ± 0	18	0.9 ± 0	20

Company 1: Substrate of barley chaff/strain *P. ostreatus* BPR-3 (Tolcayuca); Company 2: Substrate corn chaff – barley straw (50:50)/strain *P. ostreatus* PR5 (Villas de Tezontepec); Company 3: Substrate corn chaff – oat straw (50:50)/strain *P. ostreatus* L85 C11 (Progreso de Obregón); Company 4: Substrate corn chaff/strain *Pleutorus* spp. (Cuautepec de Hinojosa). The a, b, c, and d between columns indicate significant differences ($P < 0.05$); and ± is for SD; A: Non-protein nitrogen (degraded in the rumen); B1: True soluble protein (degraded in the rumen); B2: True insoluble protein (partially degraded in the rumen and intestine); B3: True insoluble protein in neutral detergent (degraded in the intestine); and C: Insoluble protein in acid detergent (linked to lignin).

Table 4. Protein Fractions of Various Substrates Degraded by *P. ostreatus* at 60 Days of Cultivation at Four Companies in Hidalgo

Company	Fraction									
	A	(%)	B1	(%)	B2	(%)	B3	(%)	C	(%)
1	2.2 ± 0.1^{a}	36	1.8 ± 0.2^{a}	29	0.4± 0^{a}	7	0.3 ± 0^{a}	5	1.4 ± 0.2^{ab}	23
2	1.5 ± 0.2^{b}	34	1.0 ± 0.2^{b}	23	0.1 ± 0^{b}	2	0.5 ± 0^{b}	11	1.3 ± 0.4^{b}	30
3	1.8 ± 0^{bc}	37	1.0 ± 0^{b}	20	0.4 ± 0^{c}	8	0.3 ± 0^{c}	6	1.4 ± 0.3^{b}	29
4	1.9 ± 0.3^{c}	28	1.1 ± 0.3^{b}	16	0.3 ± 0^{d}	4	1.7 ± 0^{c}	25	1.8 ± 0.1^{a}	27

Company 1: Substrate of barley chaff/strain *P. ostreatus* BPR-3 (Tolcayuca); Company 2: Substrate corn chaff – barley straw (50:50)/strain *P. ostreatus* PR5 (Villas de Tezontepec); Company 3: Substrate corn chaff – oat straw (50:50)/strain *P. ostreatus* L85 C11 (Progreso de Obregón); Company 4: Substrate corn chaff/strain *Pleutorus* spp. (Cuautepec de Hinojosa). The a, b, c, and d between columns indicate significant differences ($P < 0.05$); and ± is for SD.; A: Non-protein nitrogen (degraded in the rumen); B1: True soluble protein (degraded in the rumen); B2: True insoluble protein (partially degraded in the rumen and intestine); B3: True insoluble protein in neutral detergent (degraded in the intestine); C: Insoluble protein in acid detergent (linked to lignin); the a, b, c and d between columns indicate significant differences ($P < 0.05$); and ± denotes SD.

CONCLUSIONS

1. The degraded residues obtained from the cultivation of edible fungi analyzed in this study do show an increase in the content of CP and %DMD, as well as a decrease in CF, ADF and NDF; however, so that these can be used, it is necessary to standardize a single process by using the same strain, a single type of substrate, as well as reducing the use of sulfate calcium and carbonated calcium.
2. The species of *Pleurotus* used during the cultivation of edible fungi makes it possible to improve the chemical properties of the final substrate, which will depend on the type of substrate, cultivation time, interaction of the mycelium with the fibers due to the expression of extracellular enzymes, as well as other biotic and abiotic factors.
3. To use the degraded residues of the edible mushroom culture in the ruminant diet, it is necessary to carry out inclusion percentage tests according to the nutritional needs for each species and to be added with other supplements.
4. It is necessary to characterize the variations in the content of each mineral and maximum assimilable value, since they are factors that can cause serious problems in the metabolism of ruminants, in particular sheep, which prevent their full use in their diet, due to the high content of copper and other minerals.

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