Chemical Composition and Ruminal Disappearance of Maize Stover Treated with *Pleurotus Djamor*

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Abstract: A study was conducted to evaluate the effect of *Pleurotus djamor* on the chemical composition and ruminal disappearance of maize stover. Maize stover either treated or untreated with *Pleurotus djamor* was obtained from a commercial facility. Ten samples of maize stover used previously as substrate to culture edible fungus were collected randomly. The negative control group consisted of pasteurized maize stover untreated with *Pleurotus djamor*. All samples were analyzed to determine dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (C), hemicellulose (HC) and lignin (L). No differences ($P \geq 0.05$) between treatments were observed for DM, OM, CP, C, and L; however, treated maize stover ($P \leq 0.05$) showed a lower percentage of NDF as well as a lower HC value. Changes in the ruminal disappearance kinetics of the DM and NDF fractions; the potentially digestible fraction was higher in untreated maize stover ($P \geq 0.05$). The growth of *Pleurotus djamor* on maize stover changes its chemical composition by decreasing the hemicellulose content and modifying cell wall components; however, these did not improve the nutritional quality of the agricultural by-products. This suggests that *Pleurotus djamor*-treated maize stover is not ideal forage for ruminants.
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1. Introduction

Humanity is at imminent risk of hunger, two thirds of the world already suffers of it. The rate of population growth is faster than the food production capacity, the problem is further aggravated by the energy crisis and by continuous environmental degradation. Increased agro-industrial growth leads to a massive production of solid by-products, which in México are estimated to be in excess of 50 million t/year, mainly from crops such as corn, beans, rice, sorghum and barley. These in turn are burned and reincorporated into the soil (as fertilizer), used as a raw material for paper or fuel, composted, or in small percentages used for feeding animals (Guzmán *et al.*, 1987; Kuhad *et al.*, 2013).

However, the latter represent low quality forages, high in fiber, low in CP and deficient in vitamins and minerals (Mahesh and Mohini, 2013; Kholif *et al.*, 2014). All these by-products therefore have low palatability; however, some have rich soluble carbohydrate content that may represent an important energy source for ruminants (Karunanandaa *et al.*, 1995; Zadrazil, 1997). Crop residues are high in cellulose, hemicellulose, and lignin, but low in pectin and silica. An important limiting factor in using by-products for animal feeding is their low digestibility, due mainly to non-polysaccharide components such as phenolic acids (Ortega *et al.*, 1986; Kuhad *et al.*, 2013; Elghandour *et al.*, 2014). Lignin forms a ligno-cellulolic complex with some carbohydrates and proteins. This complex, especially the crystalline structure of cellulose in cell walls, is highly resistant to breakdown by enzymes, rumen microorganisms and the small intestine (Langar *et al.*, 1980; Henics, 1987). Lignin not only inhibits ruminal digestion of polysaccharides, but serves to protect other highly digestible compounds (Hadar *et al.*, 1992; Karunanandaa *et al.*, 1995). In order to increase the nutritional quality of straws and agricultural by-products, different strategies have been used to disrupt carbohydrate-lignin complexes, facilitate the access of cellulolytic microorganisms to the structural carbohydrates and improve the quality and nutritive value of straw (Montañez *et al*., 2004; Valdes *et al.,* 2015; Alsersy *et al.,* 2015). Biological agents can be used to remove lignin and increase the digestibility of low quality forages (Khattab *et al.,* 2011; Abdel-Aziz *et al*., 2015; Sharma and Arora, 2015). The basidiomycetes fungi have the capability to degrade lignin in cell walls (Khattab *et al.,* 2013; Sharma and Arora, 2015). Among these are white-rot fungi, which are capable of decomposing and mineralizing plant cell components because, during fungal colonization of a suitable substrate, the easily digestible carbohydrates are converted into simpler sugars (Khattab *et al*., 2013; Kholif *et al*., 2014). This is known as the fungus' primary metabolism. These sugars are totally consumed by the fungus and afterward secondary metabolism is initiated, which consists of the breakdown of structural carbohydrates and lignin from substrates by extra-cellular enzymes like laccase, manganese peroxidase and peroxidase (Kuhad *et al*., 2013). Because of the high annual production of agricultural residues and their low nutritional quality (Khattab *et al*., 2013), it is essential to seek new techniques to increase their nutritive value, at low energy cost and in an environmentally safe manner. These by-products should be recycled through animal feed without affecting the animals' productive performance. The objective of this study was to evaluate the effect of *Pleurotus djamur* on the chemical composition and ruminal disappearance of DM and NDF of maize stover.

2. Material and methods

All samples were collected at a commercial facility that produces fungi in Jalisco, México. The maize stover that was used as a substrate to grow the edible fungus *Pleurotus djamor* came from the southern region of Jalisco Mexico. The maize stover was pasteurized in water at 120 °C for 45 minutes and then cooled to room temperature. The pasteurized substrate was manually packaged into clean polyethylene bags 10 kg per bag, and inoculated with 250 g of the *Pleurotus djamor* strain. Culturing was carried out in an incubation room at 25–26 °C for 60 d. The fruit-bodies were harvested on days 52 and 53 after inoculation. Ten samples plastic bags of wheat straw previously used as substrate to culture edible fungi were collected randomly. The negative control group consisted of the pasteurized maize stover without treatment with *Pleurotus djamor* (MSU). Samples were transported to the Animal Nutrition Laboratory of the Southern University Center of Guadalajara University at Ciudad Guzmán, Jalisco, México. Samples were ground in a Willey mill using a 1 mm screen, then analyzed for dry matter (DM), organic matter (OM), crude protein (CP) and ash; all these determinations were based on the procedures described by AOAC (2007). Lignin, NDF and ADF were determined according to the methodologies of Van Soest (1991). All analyses were run in triplicate.

2.1.In situ degradability

In situ digestibility was determined using four 4-year old Holstein cows (625 ± 63 kg) equipped with permanent rumen cannula with a 10 cm core diameter. Cows were distributed at random in an experimental design in simple sequences of treatments. The experiment lasted 30 days, divided into two periods of 15 days each (10 days for adaptation and 5 days for sampling). The diets consisted of: maize stover treated with *Pleurotus djamor* (MST) or untreated maize stover *ad libitum* plus 1.0 kg of commercial dairy concentrate (APILECHE ULTRA®, México, México) split into two sessions (AM and PM) to ensure the greatest cellulolytic activity by the microflora in the rumen. Fresh clean water was available *ad libitum.* To determine the *in situ* digestibility of DM and ADF, we followed the procedure proposed by Vanzant *et al.* (1998). Nylon bags were used (10 x 15 cm, pore size 40 to 60 µm) with 5 g of sample. Each sample of the proposed treatments (MST and MSU) was incubated in the rumen for 8, 12, 24 and 48 h in triplicate. Additionally, for each point in time, blanks secured with nylon thread to a piece of string (30 cm long, weight 150 g) were added and left suspended in the rumen. Subsequently, the bags were removed from the rumen according to the incubation times along with the zero hour and then washed with running water under low pressure until the wash water was just as clear as before it had entered. Next, the bags of waste were dried in a circulating air oven (48 h at 60 °C). The ruminal disappearance data were analyzed using the Ørskov and McDonald (1979) model as follows: $P = a + b\,(1 - e^{-ct})$, where *P* describes the proportion (%) of material that disappeared from the bag at time *t*; *a* is the ruminal solubility, or fraction that disappeared from the bag at 0 h; *b* is the insoluble, but potentially digestible fraction (%); and *c* is the disappearance rate (%/h).

2.2. Statistical analysis

Data from the ruminal disappearance were analyzed using the NLIN procedure of SAS and chemical composition was analyzed using PROC GLM (SAS, 1999).

3. Results

No differences were observed (P>0.05) between treatments for DM, OM, CP, C, and L; however, treated maize stover (P < 0.05) showed a higher percentage of ADF, as well as lower NDF and HC values.

Table 1. Chemical composition of maize stover either untreated (MSU) or treated (MST) with *Pleurotus djamor* (%).

Component	**MSU**	**MST**
Dry matter	92.0	92.6
Organic matter	82.5	82.6
Crude protein	4.8	4.4
Neutral detergent fiber	55.9a	47.6b
Acid detergent fiber	25.7	27.2
Cellulose	33.7	32.4
Hemicellulose	29.8a	20.4b
Lignin	12.2	11.9
Ash	9.4	9.6

[a,b]Different letters following means in the same raw indicate differences at P<0.05. MSU, maize stover untreated; MST, maize stover treated with *Pleurotus djamur*.

There were differences in DM and NDF disappearance (P<0.05). The values of potentially digestible ruminal (*b*) DM and NDF were higher for MSU, while the constant of ruminal degradation (*c*) was higher for MST in the NDF fraction (P>0.05), but similar for the DM fraction.

Table 2. Ruminal disappearance of DM and NDF in maize stover either untreated (MSU) or treated (MST) with *Pleurotus djamor* (%).

In sacco disappearance	**MSU**	**MST**	**SEM**
Incubation time (h)			
8	53.68a	42.09b	1.85
12	55.67a	44.86b	1.92
24	60.49a	51.78b	1.66
48	66.45a	61.03b	1.17
DM fraction (%)			
Soluble (*a*)	35.7b	48.9a	1.98
Potentially digestible (*b*)	37.9a	23.9b	2.52
Potential degradability (*a*+*b*)	73.6	72.9	1.02
Constant of degradation (*c*)/h	0.023	0.027	0.01
NDF fraction (%)			
Potentially digestible (*b*)	24.13a	11.10b	1.2
Constant of degradation (*c*)/h	0.03b	0.06a	0.01

[a,b] Different letters following means in the same raw indicate differences at P<0.05. MSU, maize stover untreated; MST, maize stover treated with *Pleurotus djamur*; SEM, standard error of the mean.

4. Discussion

No differences were observed in DM, OM, CP or ash. Similarly, Escalona *et al.* (2001) did not find changes in DM after adding *Pleurotus florida* to a mix of molasses residues, sugarcane bagasse, and liquid residues. By contrast, Kerem *et al.* (1992) using steams of cotton, and Tripathi and Yadav (1992), Ginterová and Lazarová (1987), and Kishan *et al.* (1990) using wheat straw, all found reductions in DM after treatment with *P. ostreatus*. In addition, Yamakawa *et al.* (1992) reported reductions of 23% in DM of rice straw. Langar *et al.* (1980) also showed that during *P. ostreatus* fructification on wheat straw, OM and other cell wall components with the exception of lignin were reduced after the fungus was removed. Ortega *et al.* (1986) and Escalona *et al.* (2001) reported that ash concentration was increased 60 days after barley straws were incubated with *P. ostreatus,* possibly due to higher OM use by the fungus. Jafari *et al.* (2007) found changes in DM, OM and CP on fungal treated rice straws with different species of *Pleurotus*. In another study, cultivation of *P. pulmonarius* and *P. florida* on wheat straw resulted in greater OM digestibility for treated straw (Montañez *et al.*, 2004, 2008). Karunanandaa *et al.* (1995) also reported losses of OM in rice straw treated with white rot fungus, with these losses occurring mainly at the moment of fruit production. However, Ortega *et al.* (1986), recorded no change in CP when wheat straw was treated with *P. ostreatus* for 45 or 60 d. In contrast, Coronel and Ortega (1998) reported a higher nitrogen concentration; they attributed this increment to fungal residues left after the harvesting process. Rao and Naik (1990) used *P. ostreatus*-incubated wheat straw to feed ruminants, and found that ADFI and CP increased for *P.* ostreatus-treated straw. They also reported that some microorganisms associated with the fungus have the ability to fix nitrogen from the atmosphere, and this resulted in an increment in the nitrogen content of the treated straws.

In the present study, NDF and HC were lower for fungus-treated maize stover while ADF was similar for both treatments. Badarinaa *et al.* (2013) mentioned that cellulose, hemicelluloses, and lignin are the main sources of carbon and energy for *P. ostreatus* growth, while protein serves as the N source. Based on the results of this study, it is possible that the changes in fiber components are influenced by the substrate used for the fungus incubation, suggesting that *Pleurotus djamor* used NDF as an energy source. Gintevorá and Lazarová (1987, 1988), incubated *P. ostreatus* on wheat straw and observed that hemicelluloses and lignin were more degraded than cellulose. Hemicelluloses and lignin are two high-energy compounds that are metabolized during the growth stage of the fungus in almost the same proportion. This situation therefore, depends on the species and strains of fungus used. A significant reduction in the lignin-carbohydrate complex may increase soluble detergent fiber, and improve its quality as a feedstuff for animals. Adamovic *et al.* (1998) cultivated *P. ostreatus* on wheat straw and found, that after seeding, neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were decreased. Díaz and Sánchez (2002) found that NDF content decreased in maize straw treated with *P. ostreatus*, similar to this study. Alborés *et al*. (2006) using *P. ostreatus* strains 814, 816, *P. cornucopiae* and *P. djamor* on citrus bagasse, rice straw and a combination of both, detected decreases in neutral detergent fiber and acid detergent fiber contents.

The results of *in sacco* digestibility was higher in MSU in all times, but the DM and NDF ruminal disappearance observed in this study are similar to those in a report using *P. djamuor* where the *in vitro* digestibility of DM was similar but the NDF was higher that the control (Jafari *et al.*, 2007). Vadiveloo *et al*. (2009) reported that 25 days of treatment with *Pleurotus sajor-caju* increased the *in vitro* digestibility of rice husk. Fazaeli *et al.* (2002) observed that the digestibility of DM and OM for cattle was increased by using *Pleurotus florida* on wheat straw. White rot fungi degrades highly digestible polysaccharides and consumes relatively more lignin and hemicellulose than cellulose in its primary metabolism, and in its second metabolism degrades cellulose more than hemicellulose and lignin (Zadrazil and Kamra, 1996). For this reason, as the time of incubation increased, the cell wall components and their digestibility decreased, similar to findings in this study. Pan *et al.* (2012) observed that the effective rumen degradability of DM and OM during cultivation of the edible fungi *Pleurotus ostreatus*, *Auricularia polytricha*, *Flammulina velutipes* and *P. ferulae*, as well as the potential degradability values of *A. polytricha*, *F. velutipes* and *P. ferulae,* were significantly higher on the spent mushroom substrate. The effective rumen degradability of NDF and ADF were increased by fungal cultivation, and were also fit for use as feed for ruminants. Kim *et al.* (2011) reported that using sawdust-based oyster mushroom spent substrate, the DM disappearance rate was unchanged by fungal treatment. Díaz and Sánchez (2002) reported, that using maize straw treated with *P. ostreatus*, the proportion of DM ruminal disappearance was higher than that obtained in untreated maize straw. The potentially digestible (*b*) and constant of degradation (*c*) values were also higher after this treatment, similar to findings in this study. Although the use of fungal species improves the nutritive value of the by-products, factors such as substrate type, condition and method of cultivation as well as the fungal species selected can greatly influence the outcome. For this reason more studies should be done. The growth of *Pleurotus djamor* on maize stover changes its chemical composition by decreasing hemicellulose content and modifying cell wall components; however, this did not improve the nutritional quality of the agricultural by-products.

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