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RESEARCH

Effects of *Pleurotus sapidus* (Schulzer) Sacc. treatment on nutrient composition and ruminal fermentability of barley straw, barley rootless, and a mixture of the two

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Barley (*Hordeum vulgare* L.), and its derivatives, ranks fourth in cereal production worldwide, and the *Pleurotus* species are among the most efficient types of lignocellulolytic white-rot fungi. The objective of this research study was to evaluate the degradation of barley straw and barley rootless with an inoculum of *Pleurotus* to improve their nutritional availability as a food source for ruminants. Two experiments were conducted; the first was to determine the effects of inoculation of *Pleurotus sapidus* (Schulzer) Sacc. (PS) in barley straw (BS), barley rootless (BR), and a 75% BS and 25% BR mixture (M). The second experiment was to evaluate the same substrates *in vitro* ruminal fermentation. Barley rootless had better organic matter (OM) degradability than BS after 24 h incubation with PS. The protein content in BR was higher than in BS (P < 0.01). Enzyme activities had the highest concentration from the start of fermentation, and *in vitro* dry matter (DM) degradability in BS and BR increased after 8 and 24 d fermentation, respectively (P < 0.05). Propionic acid concentration was enhanced after 16 d fermentation in BR (P < 0.5). The use of BS combined with BR exhibited better fermentation; this result provides relevant information for integrating BR with other substrates and improving the use of straw, which can be more nutritionally available for feeding ruminants.

Key words: Barley, *in vitro* fermentation, enzyme activity.

INTRODUCTION

Different countries in the Americas, characterized by highlands of up of 1500 m.a.s.l. with long winters and severe cold, have good conditions for cultivating barley (*Hordeum vulgare* L.) Barley is a very important source of malt and food for humans and animals; 5% of the annual world production is generally reserved for seed and 51% to 70% is used directly or indirectly as animal feed, Barley straw is used as animal bedding in high technology livestock production, but also as animal feed, especially in rural areas of developing countries. Mixed cropping with vetches (*Vicia sativa* L.) is another practice to produce quality forage for grazing or cutting as hay or silage. The second most important use of barley grain is for malting; 30% of the world's barley production is for malting purposes (Dong et al., 2006; VanDeKerckhove et al., 2011). In addition to barley, wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) are also malted, but barley grain has been preferred to other grains. The reasons why barley is commonly used for malting are because its husk protects the coleoptiles during the germination process and filtering, barley grains have a firm texture, and for tradition. Ninety percent of malted barley is used for malting beer and the remainder for food substitutes (Mohammed and Getachew, 2003).

Barley rootless is a byproduct of beer that comes from germinating barley grain in the malting process where starch is hydrolyzed into sugar to obtain beer malt. Barley rootless is used to feed ruminants and non-ruminants as a source of protein, while barley straw is only used as a source of fiber and food supplement for ruminants. Physical, chemical, and mechanical processes are used to improve the nutritional quality of the straw. The chopping process is widespread but not used much due to cultural factors or lack of equipment. Applying ammonia directly to the straw was once very widespread; however, the high cost and lack of subsidies make it very unprofitable. Nowadays, new and cleaner technologies

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are being considered; therefore, biotechnologies have been incorporated that involve fungi for white rot as a biological approach to improve the nutritional quality of the byproducts (Arora and Sharma, 2009). The *Pleurotus* fungus can degrade lignin, which is a compound that limits the digestibility of structural carbohydrates contained in straw. Different publications have stated that the enzymatic mechanism of *Pleurotus* is effective in degrading complex molecules, such as lignin, and other structural compounds because of its multi-enzymatic system (Salmones et al., 2005).

In the literature, research about fungi-treated straw does not include any studies evaluating the enzymatic activities of fungi used in white rot and cultivated in substrate mixes of the malting industry. The aim of the present work was i) to study the chemical composition and production of enzyme complexes by fungi *Pleurotus sapidus* (Schulzer) Sacc. at different incubation times under solid state fermentation of barley straw crops, barley rootless, and a mixture of the two, and ii) to evaluate its influence on the degradation of DM, gas kinetics, and volatile fatty acid production on *in vitro* ruminal fermentation.

MATERIALS AND METHODS

Two experiments were conducted in this research study. The first was to evaluate the nutritional components and enzyme activity in barley straw (BS), barley rootless (BR), and a mixture of 75% BS and 25% BR (M) inoculated with *Pleurotus sapidus* (Schulzer) Sacc. for different fermentation times in solid substrate (FSS). The second was to evaluate *in vitro* ruminal fermentation of three substrates used in the first experiment.

Experiment 1. Nutritional components and enzymatic activity

This experiment was conducted at the Colegio de Postgraduados, Mexico; BS was obtained from a commercial supplier in the Municipality of Calpulalpan (Tlaxcala, Mexico) and BR from a local brewing plant (Cebadas and Maltas). Samples from each batch (four samples) and from each substrate were mixed and stored in sealed plastic bags at 4 °C.

Preparation of substrates. Barley straw was chopped to a theoretical length of 2.5 cm and BR (3.0 mm) was used without any further reduction of particle size. Portions of BS and BR were mixed in a 75:25 ratio (DM basis) with a horizontal mechanical trowel mixer to obtain a BS-BR mixture (M).

The BS, BR, and M prepared above were weighed in five plastic bags (5 kg each) with holes (≤ 2 mm). The bags were then put through the hydration and pasteurization process in metallic containers (200 L capacity, one bag per container). The process consisted of adding 4 L of tap water to each container, hydrating for 30 min and pasteurizing at 80 °C for 60 min. Bags were subsequently withdrawn from the containers and water in the bags was drained by hanging the bags in a temperature (25 °C) and humidity controlled (70% to 80%) room (3 × 6 m) for 60 min. The material in the bags was then placed on tables in the same room to cool down to 15 °C.

Treatment of substrate with *P. sapidus*. The basidiomycete *P. sapidus* culture (CP50) used in this experiment was obtained from the Prodicet laboratory (D.F., Mexico). The mycelia were maintained on 1.5% agar plates with standard nutrient liquid. To prepare inoculum, the isolate was established by mixing the mycelium block (1 cm^2) with pasteurized sorghum grain (500 g) using procedure described by Peláez-Acero et al. (2008). The culture was incubated at 25 °C for 8 d for the mycelium to grow and invade the sorghum grain.

To begin inoculation, previously prepared BS, BR, and M were weighed to obtain 20 portions of 475 g. Four uninoculated samples of each substrate were used as controls and 16 samples (of each substrate) were manually mixed with 25 g *P. sapidus* mycelium as described above to inoculate the fungus. Uninoculated *P. sapidus* (*pu*) and inoculated BS, BR, and M (500 g composite sample) were then immediately put into plastic bags (40 × 60 cm; 2 kg per bag) and sealed. All the bags for each treatment (BS, BR, and M) were prepared and subsequently placed in a room (25 ºC, 80% RH, and ≤ 20% luminosity). Four bags of each treated material (including uninoculated) were retrieved at 1, 8, 16, and 24 d after fungal inoculation. A sub-sample from each bag was dried (60 ºC for 2 d) for nutrient analysis. To determine enzyme activity and soluble protein, a 400 g sample was taken from each bag, mixed with 60 mL of sodium citrate buffer (50 mM pH 5.3), and manually stirred for 30 min in an ice bath. The mixture was first filtered through two layers of cheesecloth, and the filtrate was then centrifuged (4 °C, 12 100 g, 30 min).

Analytical procedures. The dried samples for nutrient characterization were ground and passed through a 1 mm screen and subsequently analyzed for DM, organic matter (OM), total protein (Kjeldhal), acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin according to AOAC methods (AOAC, 2005). Cellulose and hemicellulose contents were calculated as the difference between NDF and ADF, and between ADF and lignin + ashes. Soluble protein at various fermentation stages was measured at 660 nm with a spectrophotometer (Model 48000, Hach, Loveland, Colorado, USA), bovine serum albumin (BSA: 40 mg L^{-1}; Sigma-Aldrich, St. Louis, Missouri, USA) was used as a standard, and distilled water as the negative control in protein estimation.

Cellulase and xylanase activity was assayed by determining the reduction of sugar with dinitrosalicylic

acid (DNS). Carboxymethyl cellulose (CMC; Sigma-Aldrich) and oat xylan (X-0627; Sigma-Aldrich) suspended (0.5% w/v) in citrate buffer (50 mmol, pH 5.3) were used as substrates to determine cellulase and xylanase, respectively. The assay initially combined 0.1 mL of supernatant with 0.9 mL of each substrate (BS, BR, M) in capped glass tubes, which were placed in a temperature-controlled incubator (50 °C) for 5 min. The enzyme was deactivated by immediately placing the tubes into a boiling water bath for 5 min after being withdrawn from the incubator. Samples were analyzed for reducing sugar after the tubes were cooled on ice for 10 min; absorbance was measured at 540 nm against a blank prepared in the same way but with a sample of 0.2 mL of 50 mM citrate-Na citrate buffer pH 5.3 (Loera and Córdova, 2003). Laccase activity was determined by first mixing 0.1 mL supernatant with 0.8 mL citrate buffer (50 mM, pH 5.3) in a glass tube; tubes were then placed in a water bath set at 40 ºC and 0.1 mL of substrate 0.1 M 2,2'-azino-bis acid(3-ethylbenzothiazoline-6-sulfonic acid) was added. The optical density of the mixture was measured at a wavelength of 420 nm at 0 and every 30 s for 10 min.

All activities were calculated from triplicates and values were expressed in international units (IU), where 1 IU of enzyme activity is defined as the amount of enzyme required to release 1 μmol of product per minute under the given assay conditions. Activities were referred to as initial substrate dry weight (IU g^{-1} dry weight). Alternatively, when activities were lower than 1 IU, these values were expressed as mU (1000 mU = 1 IU).

Experiment 2. Determination of *in vitro* ruminal fermentation of fungal-treated BS, BR, and M

The effect of the *P. sapidus* treatment on the ruminal fermentation of BS, BR, and M was assessed with an *in vitro* gas production technique described by Ramirez-Bribiesca et al. (2011). Two cows with permanent rumen cannula were used as rumen fluid donors; they were fed 2 kg d^{-1} of concentrate (containing 16% protein) and had free access to oat straw. Animals used in this study were cared for in accordance with the standards of the Mexican Council on Animal Care (NOM-062-ZOO, 1999). Rumen fluid was collected 2 h after morning feeding by straining the rumen content collected from four different locations in the rumen-reticulum region through four layers of cheese cloth; equal amounts of rumen fluid from each cow were combined and kept at 39 °C under anaerobic conditions during transportation to the laboratory. The experiment was conducted in 125 mL amber bottles with pre-loaded substrates (500 mg per vial) to which 90 mL inoculum, a mixture of rumen fluid and mineral buffer, were added (Menke et al., 1979) in a 1:9 ratio. The bottles were flushed with CO_2 during inoculation, capped, and then placed in an incubator maintained at 39 °C. Pressure, created by the gas produced during fermentation, was measured by inserting a needle connected to a pressure gauge (INFRA metron Model 63100, Finesa, Naucalpan, Mexico) with a scale of 0-1 kg cm^{-2} in the head space of the bottle through the stopper at 2-h intervals during a 48-h incubation. The units of pressure (kg cm^{-2}) were transformed to volume ($V = (P + 0.0273)/0.0186$), and the cumulative gas production was adjusted to the logistical model described by Schofield and Pell (1995):

$$Y = v/(1 + exp(2\text{-}4 \times S \times (t - L)))$$

where Y is the total volume of gas produced (mL g^{-1} DM), v is the volume, S is the rate of gas production (mL h^{-1}), t is time, and L is lag time.

Analytical procedures. All fermentation bottles were removed from the incubator at the end of the 48-h incubation, and cultures were filtered through Whatman paper nr 54, dried at 60 °C for 48 h to constant weight, and weighed to estimate *in vitro* DM disappearance. Subsequently, 2 mL ruminal fluid from the serum bottles were centrifuged at 14 000 g for 10 min at 4 °C, and aliquots of the supernatants (1 mL) were transferred to plastic tubes each containing 0.2 mL of 25% (w/v) meta-phosphoric acid. Volatile fatty acids (VFA) in the supernatants were measured by gas chromatography (7890A, Agilent, Palo Alto, California, USA). Acids were separated with a DB-FFAP column (30 m × 0.25 mm id, 0.25 mm mesh). The carrier gas was nitrogen (N) at a rate of 0.8 mL min^{-1}. The analysis was initially isothermal for 2 min at 60 °C and then increased to 220 °C at a rate of 20 °C min^{-1} with a 280 °C detector temperature. Volatile fatty acids were identified and quantified from chromatograph peak areas by calibration with external standards.

Statistical analysis

The arrangement of treatments was factorial (3 × 5) where factors were substrate (BS, BR, and M) and fermentation time in solid substrate (*pu*, 1, 8, 16, and 24 d) with four replicates per treatment. Data were analyzed with the MIXED procedure of the SAS statistical program (SAS Institute, Cary, North Carolina, USA). A comparison of treatment means was performed by Tukey's test. The number of days of fermentation for each treatment component was analyzed by linear and quadratic contrasts. In all cases, differences among means were significant at $P < 0.05$.

RESULTS

Experiment 1. Nutritional components and enzymatic activity

Barley rootless had higher OM content ($P < 0.05$) than BS in 24 d fermentation with *P. sapidus* (Table 1). It had a linear effect over time ($P < 0.0001$) in all treatments; OM increased 2.24 g kg^{-1} from 1 to 16 d fermentation and 2.76 and 1.43 g kg^{-1} from 1 to 24 d fermentation in BR and M, respectively. The BR protein content was

Table 1. Nutritional composition of barley products inoculated with *Pleurotus sapidus* and fermented for different time periods (d).

	Barley straw (BS)							Barley rootless (BR)							Mixture (M: Barley straw and rootless, 75:25)						
	Days of fermentation							Days of fermentation							Days of fermentation						
Item	*pu*	1 d	8 d	16 d	24 d	SEM	T	*pu*	1 d	8 d	16 d	24 d	SEM	T	*pu*	1 d	8 d	16 d	24 d	SEM	T
	g kg^{-1}																				
DM	95.13	94.55	94.17	93.90	94.17	0.45	ns	95.01	94.51	94.11	94.08	93.93	0.45	ns	95.11	95.02	94.89	94.75	94.73	0.45	ns
OM	87.24	87.99	88.75	89.48	85.96	1.24	L*	87.01	87.45	88.67	88.89	89.77	1.24	L**	87.91	88.51	88.22	88.55	89.34	1.24	L*
Protein	4.01	4.12	5.10	6.06	5.58	0.13	L*	16.18	16.37	17.54	18.32	18.18	0.13	Q*	6.11	6.23	6.76	6.83	6.80	0.13	ns
ADF	51.41	50.46	50.76	47.43	47.24	0.81	Q*	39.04	38.37	37.80	35.68	32.40	0.81	L*	46.90	47.70	44.70	44.85	44.82	0.81	L*
NDF	84.95	84.65	84.24	81.59	81.35	0.48	L*	77.24	77.99	77.22	75.63	70.66	0.48	L**	83.01	82.21	80.92	80.63	80.31	0.48	L*
Cellulose	39.90	39.50	39.88	39.15	38.85	0.86	ns	30.11	30.49	30.74	29.87	26.69	0.86	Q**	38.22	38.28	36.02	36.56	36.56	0.86	Q*
Hemicellulose	35.09	34.19	34.48	34.15	34.11	1.05	ns	39.14	39.62	39.40	39.94	37.85	1.05	L*	35.11	36.51	35.22	35.74	35.49	1.05	ns
Lignin	11.07	10.96	9.88	8.28	8.38	0.25	L**	11.21	11.68	9.74	6.94	6.91	0.25	L**	8.98	9.68	8.50	8.29	8.25	0.25	L*

pu: uninoculated *Pleurotus sapidus*, DM: dry matter, OM: organic matter, ADF: acid detergent fiber, NDF: neutral detergent fiber; SEM: standard error of means, T: time effect, days of fermentation, L: significant linear effect, Q: significant quadratic effect, ns: nonsignificant.
*, ** Significant at 0.05 and 0.001 probability levels, respectively.

four times higher than in BS (4.01 *vs*. 16.18 g kg^{-1}, P < 0.01), and it improved with the inoculation of *P. sapidus* during 16 d fermentation (BS: 2.05, BR: 2.14, M: 0.72 more units of protein g kg^{-1}, P < 0.05). This result is due to the activity of the fungus when transforming existing resources, mainly fiber, structural carbohydrate polymers, and lignin. Specifically, cell wall components, represented by the amount of NDF, were degraded more rapidly in BR than BS (3.6 *vs*. 6.58 units g kg^{-1} degraded in 24 d, P < 0.05). The BS and BR mixture improved straw degradability. Degradability of nutrients analyzed in each treatment with respect to the time effect was constant, and a linear effect (P < 0.05) was mostly found in ADF, NDF, hemicelluloses, and lignin. Lignin is the principal substrate used by the fungus and its degradability in BR was higher than in BS (2.7 *vs*. 4.3 units g kg^{-1} degraded, linear effect P < 0.01); this result was associated with the amount of cellulose contained in both substrates.

Soluble protein and enzyme activities of BS, BR, and M for 1 to 24 d incubation are shown in Table 2. The percentage of soluble protein/total protein was higher in BR than BS and M (~ 41% *vs*. 34% and 32%, respectively, P < 0.05) between 8 and 16 d fermentation; subsequently, the soluble protein level dropped slightly in all treatments at 24 d fermentation (quadratic effect, P < 0.01). Conversely, the reported protein concentration was higher in BR than in BS (Table 1); these differences can be associated with soluble N released from the white fungus during its growth. However, the inoculation of *P. sapidus* in the substrate significantly improved protein content. Xylanase activity had the highest concentration and was more than 10 times from the beginning of fermentation up to 16 d. Subsequently, the xylanase concentration decreased slightly in BS and BR, or was maintained in the M treatment during 24 d fermentation (quadratic effect, P < 0.05). Cellulase activity increased linearly from baseline to 24 d fermentation in all treatments inoculated with *P. sapidus*. Surprisingly, laccase activity in all treatments was higher than xylanase and cellulase, and BS had a higher content and concentration rate than BR.

Experiment 2. *In vitro* ruminal fermentation

The *in vitro* DM disappearance (IVDMD) variables (V, S, and L) of gas production kinetics and VFA production for substrates inoculated with *P. sapidus* fermented for different time periods are shown in Table 3. There were no significant differences in all the treatments between uninoculated substrate and inoculated *P. sapidus* at 1 d fermentation (P > 0.05). However, IVDMD in BS increased after 8 d fermentation, and then decreased over time (quadratic effect, P < 0.05), while IVDMD in BR increased over time until reaching the maximum level at 24 d fermentation (linear effect, P < 0.05). Gas production decreased in BS over fermentation time (linear effect,

Table 2. Soluble protein and enzyme activity of barley products in cultures with *Pleurotus sapidus*.

	Soluble protein (mg g^{-1} dry weight)							Xylanase (IU g^{-1} dry weight)						
Treatment	*pu*	1 d	8 d	16 d	24 d	SEM	T	*pu*	1 d	8 d	16 d	24 d	SEM	T
BS	0.59	0.89	2.2	2.3	1.7	0.42	L,Q**	13.51	15.75	193.31	200.54	182.06	9.3	L**
BR	3.60	4.10	7.4	7.3	5.9	0.42	L,Q**	12.81	13.15	157.41	145.55	112.15	9.3	Q**
M	0.81	1.27	2.4	2.0	1.5	0.42	L,Q**	16.35	15.53	195.97	197.26	161.05	9.3	Q**
	Cellulase (IU g^{-1} dry weight)							Laccase (IU g^{-1} dry weight)						
Treatment	*pu*	1 d	8 d	16 d	24 d	SEM	T	*pu*	1 d	8 d	16 d	24 d	SEM	T
BS	0.88	1.51	1.87	2.59	3.19	0.18	L**	0.01	0.01	3.12	4.57	4.32	0.34	L**
BR	0.94	1.22	1.64	2.59	3.19	0.18	L**	0.04	0.03	1.77	4.37	4.70	0.34	L**
M	0.99	1.22	1.64	1.63	2.26	0.18	L**	0.13	0.11	1.69	4.11	4.49	0.34	L**

BS: Barley straw, BR: barley rootless, M: mixture of barley straw and rootless (75:25), *pu*: uninoculated *Pleurotus sapidus*, SEM: standard error of means, T: time effect, days of fermentation. L: significant linear effect, Q: significant quadratic effect.
** Significant at 0.001 probability level.

Table 3. Effect of barley straw, barley rootless, and a mixture of the two inoculated with *Pleurotus sapidus* for different fermentation periods (d) on *in vitro* DM disappearance (IVDMD), gas production, estimated kinetic parameter, and volatile fatty acids on *in vitro* ruminal fermentation for 48 h.

	Barley straw (BS)							Barley rootless (BR)							Mixture (M: Barley straw and rootless, 75:25)						
	Days of fermentation							Days of fermentation							Days of fermentation						
Item	*pu*	1 d	8 d	16 d	24 d	SEM	T	*pu*	1 d	8 d	16 d	24 d	SEM	T	*pu*	1 d	8 d	16 d	24 d	SEM	T
IVDMD, g 100 g^{-1}	50.25	52.59	55.78	53.49	52.2	0.81	Q*	61.56	60.68	71.06	71.11	71.18	0.81	L*	59.36	58.33	66.55	64.76	64.86	0.81	Q*
Gas production, mL g^{-1}	156.4	165.4	152.63	153.53	128.83	0.45	L**	173.81	169.28	185.42	190.12	178.33	0.45	Q**	183.61	190.36	177.57	177.31	164.43	0.45	L**
Lag time (L), h	7.47	7.47	7.81	7.04	8.07	1.24	ns	6.75	5.75	3.38	4.27	5.42	1.24	Q*	6.18	5.80	5.48	5.56	5.56	1.24	ns
RGP(s), h^{-1}	0.031	0.032	0.031	0.032	0.031	0.13	ns	0.032	0.031	0.032	0.032	0.03	0.13	ns	0.031	0.033	0.032	0.032	0.031	0.13	ns
Volatile fatty acids, mmol mol^{-1}																					
Acetic	69.82	68.31	67.91	68.49	70.2	0.48	Q*	67.30	68.03	65.02	63.44	70.09	0.48	Q**	68.57	69.75	69.14	68.91	70.53	0.48	Q*
Propionic	16.59	18.95	19.36	19.05	18.78	0.86	ns	19.62	20.62	22.65	23.19	18.06	0.86	Q*	19.50	19.00	19.55	19.54	18.48	0.86	ns
Butyric	13.58	12.73	12.72	12.44	11.01	1.05	L*	13.07	11.33	12.32	13.35	11.84	1.05	ns	11.92	11.24	11.30	11.54	10.88	1.05	ns

pu: uninoculated *Pleurotus sapidus*, RGP = fractional rate of gas production, h^{-1}, SEM: standard error of means, T: time effect, days of fermentation. L: significant linear effect, Q: significant quadratic effect, ns: nonsignificant.
*,** Significant at 0.05 and 0.001 probability levels, respectively.

P < 0.01). Maximum gas production was reached at 16 d fermentation in BR (quadratic effect, P < 0.01) and exhibited a shorter lag phase time for BR than BS (P < 0.05). The mixture of both substrates showed behavior that was similar to BS for the analyzed gas kinetic parameters.

There was no significant difference in VFA between uninoculated substrate and inoculated *P. sapidus* with 1 d fermentation (P > 0.05). However, acetic acid in BS, BR, and M had a quadratic effect (P < 0.05), the mean butyric acid concentration at the start in the three treatments was 68.7 mmol mol^{-1}; butyric acid decreased between 8 and 16 d fermentation and increased again up to 24 d fermentation. The butyric acid concentration in BS decreased by 1.7 mmol mol^{-1} up to 24 d fermentation (linear effect, P < 0.05); BR and M did not differ significantly between fermentation times (P > 0.05). Propionic acid concentration was enhanced to 2.6 mmol mol^{-1} at 16 d fermentation in the BR group (quadratic effect, P < 0.5); BS and M were not significantly different (P > 0.05).

DISCUSSION

Experiment 1. Nutritional components and enzymatic activity

The nutritional analysis of BS and BR before inoculation showed slightly higher NDF content, and crude protein (CP) was similar as reported by NRC (2001). Logically, differences in substrates are attributed to variations in maturity and the process of the product obtained from the malting industry. Inoculation with *P. sapidus* at different fermentation times decreased the cell wall components of the two substrates and their mixture (M). Previous studies also reported that treatment with white-rot fungi increased the nutritional value of straw (Tripathi et al., 2008; Shrivastava et al., 2010). Wheat and *Brassica* straw treated with *Pleurotus ostreatus* (Rodrigues et al., 2008) and *Trametes versicolor* (Ramirez-Bribiesca et al., 2011), respectively, exhibited an increase in CP and a decrease in organic C and the C/N ratio as compared with untreated straw. It has been suggested that the decrease in hemicelluloses can be attributed to initial consumption of carbohydrates during mycelial growth (Rodrigues et al., 2008). Peláez-Acero et al. (2008) also reported that NDF was reduced when wheat straw was treated with six fungal species. It has been previously shown that white-rot fungi, including *P. sapidus*, can degrade crop residues during solid state fermentation as a result of action by carbohydrases (cellulases and xylanases) and oxidative ligninolytic enzymes, including lignin peroxidases, manganese peroxidase, and laccase (Singh et al., 2010). Lignin removal during inoculation and fermentation in this experiment increased the susceptibility of biomass to enzymatic hydrolysis (Yu et al., 2010). This suggests that the extent of fiber degradation is related to more than the type or total amount of enzymes produced (Sharma and Arora, 2010). The effect obtained by the BS and BR mixture (M) maintained the enzymatic expression of the *P. sapidus* fungus.

The action of *P. sapidus* recorded maximum values in xylanase production from 16 to 24 d fermentation, while Xiong et al. (2005), using *Trichoderma reesei* and Membrillo et al. (2011) with *P. ostreatus,* reported an earlier xylanase activity at 4 and 12 d, respectively. However, differences in response times are attributed to the performance of the white-rot fungi on the substrate characteristics and fermentation processes. Elisashvili et al. (2008) evaluated fermentation in solid substrate (FSS) compared with submerged fermentation (SmF) using *Pleurotus* spp. and *Lentinus* spp. under several conditions that allowed expressing the production of carboxymethylcelluse, xylanase, and laccase enzymes; results varied with the type of white-rot fungi and growth conditions. These authors also reported laccase production levels of 4.1 mU g^{-1} dry weight, which is similar to those found in the present study with *P. sapidus*. Locci et al. (2008), using bran straw as a substrate with *P. ostreatus* in FSS for 62 d, showed good colonization by white-rot fungi, which allowed detecting changes in the chemical

composition of the substrate. Simultaneously, *P. sapidus* inoculated in BS and BR showed the best cellulase and laccase production between 16 and 24 d fermentation.

The results of this experiment showed adequate enzymatic production of cellulases, laccases, and xylanases, as well as protein levels for the BS and M substrates; these were similar to the response obtained by Sainos et al. (2006); they used *P. ostreatus* on barley straw and barley grain and combined both in different proportions to report that the substrate with the highest levels was the one with 50% of each substrate because barley straw increases metabolic activity and barley grain is an important nutrient reserve. In the case of the present study, BR supplies nutrients to our medium and achieves xylanase and laccase activity levels in substrate M that are similar to those of substrate BS; M is greater than BS for cellulase production levels. Barley rootless, a byproduct of the malting industry, can be mixed with barley straw and inoculated with a white-rot basidiomycete, such as *P. sapidus*; as a consequence of its growth on the substrate, this modifies its chemical composition, can be applied in feeding ruminants, andbe used in forages where the fungi is incorporated.

Experiment 2. *In vitro* ruminal fermentation

The highest IVDMD of BS occurred after 8 d fermentation, while it occurred between 8 and 24 d in BR and increased linearly; this degradation basically depended on the amount of the wall and cell content of each substrate. Barley rootless had lower cell wall content, which resulted in the greatest attack of *P. sapidus* on the substrate. Another study has also shown that this strain of *P. sapidus* is able to degrade noncellulosic polysaccharide components of plant cell walls because it produces a variety of other polysaccharidases, including xylanase and pectinase (Peláez-Acero et al., 2008). This can account for the ability of *P. sapidus* to digest NDF from BS and BR. The ability of this white-rot fungus to degrade NDF depended on both the substrate type and applied fermentation time. Okano et al. (2006), using *P. eryngii* in sugar cane bagasse, report that the proportion of DM and residual lignin in the substrate decreases, and consequently, its digestibility *in vitro* and *in vitro* gas production increase at 48 h. In the present study, results were similar to those reported by Okano et al. (2006). Getachew et al. (2004) evaluated *in vitro* gas production of 12 feed resources and found that dry distillery grains had the highest gas production at 48 h fermentation. In contrast, BS produced more gas in the control group than groups inoculated with *P. sapidus*, and BR had higher gas production at 16 d fermentation. Peláez-Acero et al. (2008), when using *P. sapidus* in sugar cane, found values of maximum gas volume (V) between 294.73 and 155.69 mL g^{-1} DM, a lag phase (L) between 0.31 and 3.64 h, and gas production rate (S) between 0.0248 and 0.0364 mL h^{-1}. In the present study, V varied between 219.67 and 128.83 mL g^{-1} DM, L fluctuated between 1.98 and 9.07 h, and S between 0.029 and 0.032; values were similar in both studies. Barley rootless induced a higher proportion of propionic acid (Getachew et al., 2004; Mäntysaari et al., 2007). Specifically, the treatment with *P. sapidus* decreased the proportion of acetic acid and increased propionic acid compared with the untreated substrate ($P < 0.05$) in which the acetic-propionic acid relationship was closer. Ramirez-Bribiesca et al. (2011) report a reduction in acetic acid and an increase in propionic acid from the effect of the action of exogenous enzymes; according to these authors, it has an impact on the degradation of the feed and the final products of ruminal fermentation, which improves nutrient use efficiency. When the fibrolytic and amylase enzymes of *Trichoderma viride* (xylanase), *Aspergillus niger*, and *Trichoderma viride* (Giraldo et al., 2008) acted on a mixture of grass straw and concentrated feed in proportions of 0.7:0.3, 0.5:0.5, and 0.3:0.7, they increased true DM digestibility , production of acetic and propionic acids, total volatile fatty acids, and gas after 8 h of *in vitro* ruminal fermentation, but the effects disappeared at 24 h incubation, while degradation of the substrate continued to be higher. These results show that the fungal species and type of substrate determine the changes in the substrate structure and composition, which can be beneficial or negative for their use at the ruminant consumption level.

CONCLUSIONS

The present study demonstrated the potential of using of *Pleurotus sapidus* enzymes in straw to modify the chemical composition and perhaps facilitate the bacterial function on and in the straw. The treatment of *P. sapidus* in a mixture of barley straw and barley rootless (75:25) increased lignin solubility and reduced NDF levels; the combination synergistically improved ruminal microbial digestion of straw. This result provides relevant information for integrating barley rootless with other substrates and improving the use of barley straw in feeding ruminants.

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