Effect of White-rot Fungal Treatments on the *in Vitro* Rumen Degradability of Two Kinds of Corn Stover

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The capacities of white rot fungal treatments to degrade lignocellulose and further to improve the rumen degradability were examined in two kinds of corn stover (CAU3138 and ZN01). A total of seven fungi were evaluated according to their growth rates and selective values (SV) on both stovers under 28 °C for 28 days. Then, three selected fungi were evaluated by their *in vitro* gas production (IVGP) as a measure of rumen fermentation capacity. The results showed that *Irpex lacteus*, *Pleurotus ostreatus*, and *Pleurotus cystidiosus* had high speeds of colonization and high SV on both stovers. The IVGP of the ZN01 treated with any of the three fungi was lower than that of the raw stover, and the IVGP of the CAU3138 treated with *P. ostreatus* and *P. cystidiosus* had no significant change ($P > 0.05$). However, the IVGP of the CAU3138 treated with *I. lacteus* was significantly increased ($P < 0.05$) and further increased as the treatment time was prolonged. This study indicated that SV was not always a good predictor for the performance of the fungus. Here, *I. lacteus* performed best, regarding fungal growth and IVGP of the CAU3138.

Keywords: White-rot fungi; Corn stover; Selective value; In vitro gas production; Acid detergent lignin degradation; Irpex lacteus

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INTRODUCTION

Corn stover is an attractive renewable source of roughage for ruminant animals. In China, approximately 300 million tons of corn stover are produced annually (Chen *et al.* 2017). However, corn stover has a low degradability. Consequently, most of the corn stover has been abandoned in the field or burnt directly, heavily polluting the environment. Within corn stover cell walls, acid detergent lignin (ADL), a large group of recalcitrant aromatic polymers bonded with hemicellulose by ether and ester linkages, forms a matrix that tightly surrounds the cellulose. This complex lignocellulosic structure inhibits the fermentation process by rumen microbes and hydrolyzation by cellulose (Van Kuijk *et al.* 2015b). Therefore, it is necessary to disrupt the covalent bonds between ADL and hemicellulose *via* treatment to release the cellulose from this matrix and enhance the feed utilization rate of corn stover. Moreover, development and utilization of corn stover in animal husbandry have become important for sustainable agriculture development.

Biological treatment, especially white-rot fungal treatment, has been regarded as an environmentally friendly and potentially economically viable alternative to physical and chemical treatments to improve the *in vitro* rumen degradability of the lignocellulose biomass for the ruminants (Tuyen *et al.* 2013; Van Kuijk *et al.* 2015a). However, white-rot fungi have different abilities and methods of degrading lignocellulose, and some take a relatively long time to germinate their mycelium. Moreover, white-rot fungi consume a major portion of cellulose and hemicellulose as part of the fungus' growth. For example,

although *Phanerocheate chrysosporium* is a well-known white-rot fungus that effectively degrades ADL, it is a non-selective fungus that consumes large quantities of cellulose and hemicellulose during its degradation of lignocellulosic biomass (Syed and Yadav 2012). Therefore, it is necessary to select fungi with high growth speeds that can inhibit mould and bacterial contamination (Tirado-González *et al.* 2016). In doing so, one should use a selective value (SV; ratio of ADL degradation to cellulose degradation) to screen fungi that can selectively degrade the corn stover (Tuyen *et al.* 2012; Saha *et al.* 2016). In contrast, some researchers have reported that moderate cellulose degradation can increase glucose recoveries because increased glucose recovery also contributes to the degradation of ADL (Salvachúa *et al.* 2011; Niu *et al.* 2018). That is to say, treatments with the highest ADL loss, rather than selective ADL degradation, have the greatest potential for increasing the nutritional value of the feed (Rahman *et al.* 2011). Before now there have been no certain conclusions about whether or not SV is a crucial parameter in evaluating the nutritional value of corn stover with white-rot fungal treatment. Thus, the role of SV in improving the rumen degradability of corn stover with fungal treatment should be further examined. To study the improvement in rumen degradability by white rot fungal treatment. The *in vitro* gas production (IVGP) technique was used. Menke *et al.* (1979) reported a high correlation between gas production *in vitro* and *in vivo* apparent digestibility. The IVGP technique has been widely used for evaluation of nutritive value of feed.

In the present study, seven species of fungi were used on corn stovers CAU3138 and ZN01. The CAU3138 corn stover is from the grain and forage corn variety that is tall and strong with higher ADL content. The ZN01 corn stover is from the waxy corn variety and is short and weak with lower ADL content. The objectives of the present study were: (1) to examine the differences between seven fungal species on selective ADL degradation in the corn stovers CAU3138 and ZN01; (2) to employ the IVGP technique to compare the ability of the selected fungi on the improvement of the nutritional value of CAU3138 and ZN01; and (3) based on the IVGP results, to further study the effects of two different particle sizes (1 mm and 2 cm) with a prolonged treatment time of 42 days on CAU3138 and the optimization of these parameters to obtain a high *in vitro* rumen degradability.

EXPERIMENTAL

"Experiment 1" was designed to preliminarily screen for the fungal strains with high growth rates and SV values. Then, the *in vitro* rumen degradabilities of the two kinds of corn stovers treated with the preliminarily screened fungi, as measured by the IVGP technique, were compared. Based on the results of IVGP in Experiment 1, Experiment 2 was designed to optimize the screened fungus treatments of corn stover CAU3138 with an extended treatment time and enlarged particle sizes.

Materials

Substrate preparation

Corn stover CAU3138 (approximately 110 d from sowing to harvesting) is from the grain and forage corn cultivar; corn stover ZN01 (approximately 90 d from sowing to harvesting) is from the waxy corn variety. Both stovers were collected from local farmlands at Baoding City, Hebei Province, China. The whole aboveground plant was initially air-dried at room temperature for one month. Both feedstuffs were chopped into average particle sizes of 2 cm in length. A portion was milled in RT-34 hammer mill (Rong Tsong

Precision Technology Co., Taiwan). and passed through a 1-mm screen. Both processed corn stovers were then sealed in plastic bags and stored at room temperature for further use. The chemical compositions of CAU3138 and ZN01 are shown in Table 1.

White-rot fungal strains and spawn preparation

The three white-rot fungal strains used in the present study (*Phanerochaete chrysosporium* (CGMCC-5.776), *Irpex lacteus* (CGMCC-5.809), and *Pleurotus ostreatus* (CGMCC-5.374)) were purchased from the China General Microbiological Culture Collection Center (CGMCC) in Beijing, China.

Table 1. Chemical Compositions of the Raw and Autoclaved CAU3818 and ZN01

Composition (g/kg DM)	CAU3818	Autoclaved CAU3818	ZN01	Autoclaved ZN01
NDF	742.9 ± 5.90	760.8 ± 2.52	627.8 ± 8.41	632.0 ± 3.33
ADF	462.6 ± 4.22	492.3 ± 3.00	350.6 ± 2.99	348.5 ± 2.87
ADL	86.9 ± 1.68	104.0 ± 4.18	28.22 ± 1.76	49.1 ± 0.22
NDS	257.1 ± 5.90	239.2 ± 2.52	372.2 ± 8.41	368.0 ± 3.33
Cellulose	375.6 ± 5.90	388.3 ± 1.18	322.4 ± 2.77	299.4 ± 2.56
Hemicellulose	280.4 ± 1.68	268.5 ± 0.48	277.2 ± 5.41	283.5 ± 4.91
CP	52.8 ± 1.00	61.3 ± 0.38	99.0 ± 0.89	115.3 ± 0.77
WSC	19.8 ± 1.33	20.8 ± 1.07	58.2 ± 1.74	61.3 ± 2.77
Ash	101.0 ± 3.09	98.4 ± 1.20	61.0 ± 0.98	58.4 ± 4.33

Note: NDF- neutral detergent fiber; ADF- acid detergent fiber; ADL- acid detergent lignin; NDS- acid detergent soluble; CP- crude protein; WSC- Water soluble carbohydrate; data are mean ± standard error

In addition, the following four *Basidiomycete* strains were obtained from the Shangzhuang Mushroom Breeding Center (Beijing, China), isolated in the authors' laboratory, and identified *via* rDNA internal transcribed spacer (ITS) sequence analysis. These strains were: *Pleurotus cystidiosus* (accession number: KY828220), *Gymnopus perforans* (KY848622), *Flammulina velutipes* (KY828221), and *Pleurotus ferulaginis* (MF067417). All these *basidiomycete* strains were maintained in the laboratory on Potato Dextrose Agar (PDA) slants at 4 °C. Initial cultures of these fungi were developed on PDA plates at 28 °C until mycelium covered the entire agar plates. A total of 20 pieces of colonized agar cultures (approximately 1 cm^2) of *I. lacteus* were separately added to sterilized wheat grains and incubated at 28 °C until all grains were colonized by mycelium. The spawns were kept at 4 °C until further use.

Methods

Fungi treatments of 1-mm corn stover CAU3138 and ZN01 for 28 days (experiment 1)

The sterilization and fungal inoculation processes were performed on 1-mm substrates CAU3138 and ZN01 according to Zuo *et al.* (2018). The inoculated substrates and the control (autoclaved but un-inoculated substrates) were incubated at 28 °C for 7, 14, 21, and 28 days in an air-conditioned chamber with 85% relative humidity. Triplicates of the 1-mm substrates treated with individual fungi plus the petri dishes were taken after the elapsed time, weighed, and oven-dried at 65 °C until constant weight for dry matter (DM) loss determination.

In vitro gas production technique

In vitro gas production was performed according to the procedure described by Menke *et al.* (1979). In summary, rumen fluid was collected in the morning (before feeding) from three 3-year old rumen-fistulated Angus bullocks fed a corn silage and wheat straw-based diet at the China Agricultural University Beef Cattle Breeding Center (Beijing, China). The samples were then immediately transferred to the laboratory in a thermos bottle. The fresh rumen fluid was mixed with a buffer solution in a 1:2 (v/v) ratio under a continuous flux of CO_2. Approximately 220 mg of the oven-dried corn stover substrates (already treated with each fungus) was weighed into each of the three 100-mL calibrated glass syringes. The glass syringes were pre-warmed to 39 °C, then 30 mL of mixed culture medium were pipetted with an automatic pump into each glass syringe, and then they were incubated in a water bath at 39 °C. A control was included that contained corn stover that had not been treated with any of the fungi. A blank glass syringe only contained 30 mL of mixed culture medium. All the cultures had three replicates. The gas production was manually recorded at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84, and 96 h, calculated by subtracting the volumes of gas in the blank calibrated syringes, and expressed on an OM basis.

Optimization of I. lacteus treatment of 1-mm and 2-cm CAU3138 Stover for 42 days (experiment 2)

The methods used in experiment 2 were the same as those used in experiment 1. The first part of experiment 2 investigated the effects of an extended treatment time (42 days) and the use of a smaller CAU3138 particle size (1 mm) on IVGP by *I. lacteus*. The second part of experiment 2 was followed as: the moisture content of the 2-cm CAU3138 particles was also adjusted to 70% by adding 642 mL distilled water to 300 g air-dried corn stover (DM 942 g/kg). After thorough mixing, 100 g substrates were weighed in polypropylene bags and sterilization and fungal inoculation processes were performed according to Van Kuijk *et al.* (2015b). The substrate was aseptically inoculated with 4 g of previously prepared wheat spawn of *I. lacteus*. The bags of inoculated CAU3138 along with the control were incubated in an air-conditioned chamber at 28 °C for 7, 14, 28, and 42 days. The samples of 2-cm CAU3138 (with bags) were taken at the specified times, weighed, and oven-dried at 65 °C until constant weight; then, the DM of the 2-cm CAU3138 particles was ground to 1 mm and preserved for chemical composition determination and IVGP measurement. All of the treatments were performed in triplicate.

Chemical analysis

The neutral detergent fibre (NDF) content was analyzed by the modified method of Van Soest *et al.* (1991) with the addition of a heat stable amylase but without the addition of sodium sulphite. The acid detergent fiber (ADF) content and ADL content were analyzed *via* the method of Van Soest (1973). Neutral detergent solute (NDS) content was calculated by subtracting the NDF from 1000. The hemicellulose (HC) content was calculated as the difference between the NDF and ADF, and the cellulose (Cell) content was calculated as the difference between the ADF and ADL. The water soluble carbohydrate (WSC) content was analysed using the anthrone-sulphuric acid colorimetry method (Ning *et al.* 2017). The crude protein (CP) content of the substrates was tested according to the Kjeldahl method. Ash content was determined by combustion for 3 h at 550 °C in a muffle furnace according to Van Kuijk *et al.* (2015b).

Statistical Analysis

The results of SV, chemical loss analysis, and IVGP data of the fungal treatment of each substrate were subject to a generalized linear model (GLM) analysis in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) software. The following model was used,

$$Y_{ij} = \mu + \alpha_i + \omega_{ij} \quad (1)$$

where Y_{ij} is the observation j in treatment i, μ is the overall mean, α_i is the fixed effect of treatment i, and ω_{ij} is the random error.

The effect of incubation time and particle size on the detergent fiber composition and IVGP of CAU3138 was tested using the generalized linear model (GLM) analysis in SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). The following model was used,

$$Y_{ij} = \mu + \alpha_i + \beta_j + \omega_{ij} \quad (2)$$

where Y_{ij} is the observation at incubation time i, μ is the overall mean, α_i is the fixed effect of incubation time i, β_j is the fixed effect of particle size j, and ω_{ij} is the random error.

RESULTS AND DISCUSSION

Seven Fungi Treatment of CAU3138 and ZN01

All fungi used in the present study were able to grow on CAU3138 and ZN01. In the early stage of incubation, rapid growth of *P. ostreatus*, *I. lacteus*, *P. chrysosporium*, and *P. ferulaginis* on the corn stover CAU3138 was observed. After two weeks, mycelium of the above four fungi almost covered all of the CAU3138 substrates and *P. cystidiosus* began to grow quickly at this time. For *F. velutipes*- and *G. perforans*-treated CAU3138, very limited amounts of mycelium were visually observed. Different from CAU3138, all of the fungi were able to grow rapidly on the ZN01 and almost covered the entire petri dishes during the initial two weeks.

Table 2. Component Loss (%) and Selectivity Values (ADL Degradation/ Cellulose Degradation) of 1-mm Corn Stover CAU3138 and ZN01 After 28 Days Incubation with Seven White-rot Fungi

White-rot Fungi	CAU3138					ZN01				
	DM	ADL	HC	Cell	SV	DM	ADL	HC	Cell	SV
	(%)					(%)				
I. lacteus	17.9b	33.0b	49.5b	21.7d	1.52c	31.6b	40.0b	41.8b	28.4b	1.41c
P. ferulaginis	17.9b	22.4d	41.6c	26.2c	0.85d	23.2c	11.3f	30.5d	19.4d	0.58f
P. ostreatus	13.9c	26.4c	36.0d	16.9e	1.56b	20.9d	17.9e	36.5c	12.3e	1.45b
P. chrysosp	50.1a	57.1a	80.7a	73.4a	0.78e	46.3a	47.8a	43.3a	45.0a	1.06e
F. velutipes	17.3b	3.78e	22.7f	33.4b	0.11g	23.2c	23.1d	12.4g	23.0c	1.00e
P. cystidiosus	12.2d	27.7c	26.9e	10.6f	2.61a	21.3d	17.8e	16.9f	10.0f	1.78a
G. perforans	9.73e	2.56e	22.0f	11.5f	0.22f	20.8d	30.3c	20.4e	22.9c	1.32d
Treatment	**	**	**	**	**	**	**	**	**	**

Note: *P. chrysosp-Phanerochaete chrysosporium*; Means of each fungus treatment within a column that are followed by the same letter are not significantly different from each other at $P < 0.05$; **- $P < 0.001$; DM- dry matter; ADL- acid detergent lignin; HC- hemicelluloses; Cell- cellulose

It was obvious that the ZN01 had higher nutritional value than the CAU3138 (Table 1). The cellulose and ADL content of the ZN01 was lower than that of CAU3138; moreover, the NDS content of ZN01 was higher than that of CAU3138 and its WSC content was almost three-fold that of the CAU3138. All fungi showed a net loss in DM, cellulose, hemicellulose, and ADL when on the 1-mm CAU3138 and ZN01 substrates (Table 2). For both stovers, the incubations with *P. ostreatus*, *P. cystidiosus*, and *G. perforans* showed the lowest losses of DM, whilst the incubations with *P. chrysosporium* showed the highest losses of DM ($P < 0.01$). The highest loss of ADL in the CAU3138 and ZN01 was due to *P. chrysosporium* treatment, followed by *I. lacteus* treatment ($P < 0.01$). The *P. chrysosporium* and *I. lacteus* incubations also resulted in higher losses ($P < 0.01$) of hemicellulose in both stovers than other fungi, yet losses of cellulose due to *I. lacteus* treatment were much lower than *P. chrysosporium* treatment. *Pleurotus ostreatus* consumed a small percentage of cellulose but a relatively large proportion of hemicellulose of both stover. However, incubation with *P. cystidiosus* led to the lowest loss of cellulose for both stovers ($P < 0.01$). In this study, after 28 days of treatment, the SV of CAU3138 treated by all seven strains varied from 0.11 to 2.61, while the SV of ZN01 varied from 0.58 to 1.78 (Table 2). The CAU3138 and ZN01 samples treated with *P. ostreatus*, *P. cystidiosus*, and *I. lacteus* were completely covered by their mycelium after 28 days and showed higher SV (following the order: *P. cystidiosus* > *P. ostreatus* > *I. lacteus*) after 28 days of treatment. Therefore, *P. ostreatus*, *P. cystidiosus*, and *I. lacteus* were selected for the further study.

In vitro Gas Production

Raw CAU3138 corn stover showed an IVGP value of 177.9 mL/g OM, while the IVGP of the 1-mm CAU3138 control was 199.3 mL/g OM, making this a significant increase compared with the raw material ($P < 0.01$). The gas production kinetic parameters estimated using Eq. 1 revealed that the rate of gas production was significantly ($P < 0.01$) improved after 28 days of treatment of the 1-mm CAU3138 with *I. lacteus* and *P. cystidiosus*. However, only the 1-mm CAU3138 treated with *I. lacteus* for 28 days had significantly ($P < 0.01$) greater asymptotic gas production, which resulted in an increased ($P < 0.05$) IVGP of 66.8 mL/g OM (33.5% increase). There was no increase in total IVGP for the 1-mm CAU3138 treated by *P. ostreatus* or *P. cystidiosus* (Fig. 1a). Raw ZN01 corn stover had an IVGP value of 278.3 mL/g OM. The IVGP of the ZN01 control was 273.0 mL/g OM; thus, autoclave sterilization had no significant effect on the gas production of ZN01 ($P > 0.05$). Moreover, IVGP decreased ($P < 0.01$) after the three fungi treatments with lower asymptotic and rate of gas production than the control of ZN01 (Fig. 1b).

When the treatment time was extended to 42 days for the 1-mm CAU3138, IVGP for the sample treated with *I. lacteus* increased significantly ($P < 0.01$) (Fig. 1a and 1c). For the CAU3138 stover with a 2-cm particle size, IVGP of the sample treated with *I. lacteus* did not increase compared with the 1-mm stover treated for the same periods (Fig. 1a and 1d). Interestingly, the smaller particle size was beneficial for the gas production of the *I. lacteus* treatment.

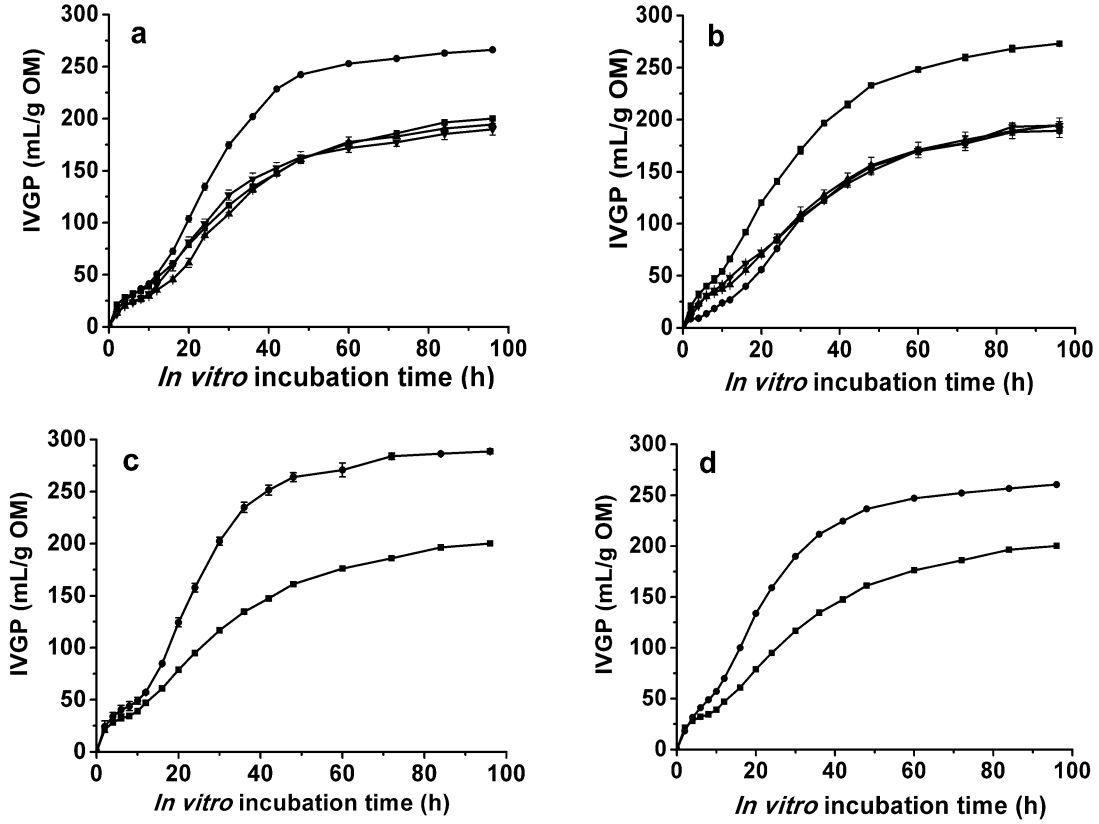

Fig. 1. Results of IVGP measurements after the selected fungi treatment of corn stover compared with the control (autoclaved but un-inoculated substrates); (a) Three fungi treatment of 1-mm CAU3138 for 28 days, (b) Three fungi treatment of 1-mm ZN01 for 28 days, (c) *Irpex lacteus* treatment of 1-mm CAU3138 for 42 days, (d) *Irpex lacteus* treatment of 2-cm CAU3138 for 42 days; (■) Control, (●) *Irpex lacteus*, (▲) *Pleurotus ostreatus*, (▼) *Pleurotus cystidiosus*; error bars indicate standard deviations, $n = 3$

Chemical Analysis of *I. lacteus* Treatment of the 1-mm and 2-cm CAU3138 for 42 Days

As no improvement in the IVGP of the ZN01 stover treated by the three selected fungi (relative to the control) was observed, no further studies of ZN01 were conducted. Table 3 shows the changes of detergent fiber and CP of CAU3138 during the 42 days of incubation with *I. lacteus*. This fungus caused a decrease ($P < 0.05$) in ADL concentration (g/kg DM) and the absolute amount of ADL (g) of CAU3138 from 7 days on; the content and absolute amount of hemicellulose also decreased with prolonged treatment times. The *I. lacteus* caused a higher loss of ADL (43 g/kg DM) in the 1-mm CAU3138 compared to the control, but it also caused a high loss of hemicellulose (106 g/kg DM). After 42 days of incubation, the ADL content of the 2-cm CAU3138 was lower ($P < 0.05$) than that of the 1-mm particles. However, the cellulose content of the 2-cm CAU3138 treated by the fungus was also lower than the 1-mm CAU3138 ($P < 0.05$). Even more notable, the absolute amount of cellulose loss was significantly larger than it was for the 1-mm CAU3138 treated with the fungus ($P < 0.01$). The CP content usually indicates the nutritional value of a feed and, in this study, the CP content increased significantly after the fungus treatment of CAU3138 with the prolonged treatment time ($P < 0.01$). However, the absolute amount of CP only increased by a small amount from the prolonged fungus

treatment time. Enlarging the particle size improved the absolute amount of CP as well as the CP content for the sample treated by *I. lacteus*.

Table 3. Chemical Composition of 1-mm and 2-cm Corn Stover CAU3138 Over a 42-Day Period of Treatment with *Irpex lacteus*

Unit		Concentration (g/kg DM)		Absolute Amount (g)	
Particle size		1 mm	2 cm	1 mm	2 cm
Component	Day				
ADL	0	104^{c}	104^{b}	104^{b}	104^{a}
	7	131^{a}	110^{a}	114^{a}	96.6^{b}
	14	111^{b}	89.1^{c}	92.9^{c}	86.8^{c}
	21	89.6^{d}	87.0^{c}	73.8^{d}	71.6^{d}
	28	83.5^{d}	71.1^{d}	68.6^{d}	55.6^{e}
	42	61.0^{d}	58.0^{e}	61.3^{e}	48.4^{e}
SEM		2.96	0.97	2.64	3.78
P-value		**	**	**	**
HC	0	269^{a}	269^{a}	269^{a}	269^{a}
	7	213^{b}	230^{b}	185^{b}	207^{b}
	14	190^{c}	203^{c}	159^{c}	181^{c}
	21	174^{d}	193^{d}	144^{d}	162^{d}
	28	168^{d}	182^{e}	138^{d}	146^{e}
	42	163^{d}	171^{f}	128^{e}	134^{f}
SEM		3.86	3.32	3.59	3.44
P-value		**	**	**	**
Cell	0	388^{a}	388^{a}	388^{a}	388^{a}
	7	385^{a}	362^{b}	333^{b}	325^{b}
	14	377^{b}	361^{b}	317^{c}	318^{bc}
	21	375^{bc}	360^{b}	309^{cd}	308^{c}
	28	370^{c}	359^{b}	304^{d}	284^{d}
	42	364^{d}	360^{b}	302^{d}	283^{d}
SEM		2.33	3.28	3.66	5.25
P-value		**	*	**	**
CP	0	61.3^{f}	61.3^{e}	61.3^{b}	61.3^{e}
	7	65.7^{e}	69.2^{d}	57.7^{d}	63.0^{d}
	14	70.0^{d}	72.4^{d}	59.0^{c}	63.8^{c}
	21	72.0^{c}	77.1^{c}	59.3^{c}	64.1^{c}
	28	74.5^{b}	81.7^{b}	61.3^{b}	65.4^{b}
	42	76.7^{a}	85.5^{a}	62.5^{a}	67.5^{a}
SEM		0.69	1.34	0.138	0.168
P-value		**	**	**	**

Note: $^{a\ to\ f}$ Means of each fungus treatment within a column followed by the same letter are not significantly different from each other at $P < 0.05$; **- $P < 0.001$; *- $P < 0.05$; ADL- acid detergent lignin; HC- hemicelluloses; Cell- cellulose; CP- crude protein

In this study, the fungal strains that grew rapidly and could inhibit mould and bacterial contamination were selected (Tirado-González *et al.* 2016). The strains of *P. ostreatus* and *I. lacteus*, which displayed high growth speeds, presented lower rates of mould contamination indicating that these fungi may have had bioactive compounds that acted as antimicrobials (Chaudhary and Tripathi 2015). Higher SV indicate relatively low cellulose losses during the fungal treatment process; an SV of 1.0 means that lignin and cellulose were equally lost (Zhang *et al.* 2007; Saha *et al.* 2016). There are several reports on using SV to screen for the fungi best at degrading lignocelluloses for various biofuel applications (Zhang *et al.* 2007; Wan and Li 2010; Saha *et al.* 2016). However, SV was

employed in this study as an initial prediction of which fungi would be best able to degrade corn stover for the use of ruminant feed, although the results indicated that SV was not reliable to screen the fungi for improvement of the *in vitro* rumen digestibility of corn stover.

The ADL content of CAU3138 was almost three-fold higher than that of ZN01 in the current study. The ADL is one of the main components of plant cell walls that limits rumen microorganism utilization of the cellulose and hemicellulose (Van Kuijk *et al.* 2015a). Therefore, the likely reason that the IVGP of CAU3138 and ZN01 treated with the same fungi showed different results was the corn stovers' different ADL contents. Moreover, Tuyen *et al.* (2013) ever reported that nutritional value improvements by some fungi treatments linearly with ADL content increased in substrates. In that study, four species of white-rot fungi treatments of rice straw, maize stover, oil palm fronds, and sugarcane bagasse were compared, and it was revealed that, for a poorly lignified feedstuff, nutritional value is not improved by treatment with *Ceriporiopsis subvermispora*, *Lentinula edodes*, *Pleurotus eryngii*, or *Pleurotus ostreatus*. It was confirmed in this study that the total IVGP after fungal treatment of the (lower ADL content) corn stover ZN01 was not improved compared with the control. However, fungal treatment of the higher ADL content CAU3138 resulted in a large increase of IVGP after treatment with *I. lacteus*, which indicated that *I. lacteus* has the potential to improve the nutritional value of corn stover with higher ADL content. In addition, the higher SV of *P. cystidiosus* treatment but lower ($P < 0.01$) gas production indicated that there was no close relationship between SV and IVGP. This was inconsistent with the screening method, which was based on the SV applied in the biofuels industry, and different from the conclusions that there is a close relationship between the increase in gas production and the lignin to cellulose loss ratio for white-rot fungal treatment of wheat straw reported by Tuyen *et al.* (2012). However, this result was consistent with Niu *et al.* (2018), who reported that *P. ostreatus* had a higher loss ratio of ADL to cellulose than *I. lacteus* but also had a lower gas production. The differing results were likely due to the patterns of attack on lignocellulosic biomass by fungi are different (Wan and Li 2010), and, possibly the diverse characteristic of the substrates.

The effect of the duration of the *I. lacteus* treatment period on IVGP (for CAU3138 stover) indicated that prolonging the treatment by this fungus could increase the IVGP. However, this might have been due to the significant decrease in ADL content between days 28 and 42 by this fungus, thus improving the availability of fermentable nutrients in the stover for rumen microbes. Tuyen *et al.* (2013) reported that after 6 weeks *C. subvermispora* and *P. eryngii* increased the total gas production of corn stover more than the autoclaved but un-inoculated substrates (control) and a sample with a 3-week incubation period, and that the samples treated with *L. edodes* and *P. ostreatus* had less gas production than the control. This was possibly because the ADL content of the corn stover used in that study was less than that of the CAU3138 used in the present research. The composition of gas production *in vitro* had been reported by Tuyen *et al*. (2013), who indicated that the use of *C. subvermispora* to improve the nutritive value of the oil palm fronds will potentially result in a decreased methane emission. Therefore, the composition of gas production *in vitro* in this study should be analyzed in the future to make clear whether *I. lacteus* treatment can decrease the methane emission.

The particle size of the substrate had differing effects on the fungal treatments of the corn stovers. Salvachúa *et al.* (2013) reported that digestibility was significantly improved as particle size was reduced for *I. lacteus*-treated wheat straw cultures. This is

explained by the fact that with decreasing particle sizes, a larger surface to volume ratio is obtained, which makes the carbohydrates more accessible. Gómez *et al.* (2011) demonstrated this when *Trametes sp.* was grown on smaller particles of corn stover and an expression of cellulases and xylanases was increased. However, it is not practical to apply such small particle sizes of corn stover in the ruminant feed. Moreover, Van Kuijk *et al.* (2016) has also reported that particle size did influence *L. edodes* treatments of wheat straw. The *L. edodes* treatment of 2-cm wheat straw resulted in a more selective delignification and a higher IVGP value than the smaller particles. Furthermore, to reach the carbohydrates in large particles, the fungi have to degrade more ADL than they would for smaller particles, which explains why the 2-cm particles CAU3138 resulted in a lower ADL after *I. lacteus* treatment in this study. The IVGP results of this study showed that a particle size of 2 cm had a negative influence on *I. lacteus* treatment of CAU3138. This was probably because the cellulose content also decreased after the fungus treated the larger particle size of the stover. However, the IVGP still increased compared with the control. Nonetheless, the effects of *I. lacteus* treatment on other particle sizes of CAU3138 between 1-mm and 2-cm should be further studied to optimize the appropriate particle size.

The ADL content was slightly increased in the initial stage of fungal treatment of 1-mm and 2-cm CAU3138 on day 7 mainly due to the heavy loss of other components. This was similar to the wheat straw treatment results with *L. edodes* reported by Van Kuijk *et al.* (2015b). Prolonging the treatment time further degraded the ADL content of 1-mm CAU3138, which was consistent with the study by Xu *et al.* (2010) but would be inadvisable for the utilization of corn stover because there was too great of a loss in DM. The cellulose loss seen in the *I. lacteus* treatment of 2-cm CAU3138 was larger than that from the 1-mm CAU3138, which is disadvantageous for the effective utilization of the corn stover; an increase in CP content suggests an increase of nitrogen in the treated corn stover. This result needs further clarification as it is generally accepted that white-rot fungi are unable to fix atmospheric nitrogen from the air (Millbank 1969). However, the enrichment of the CP content of CAU3138 was consistent with the research of Nayan *et al.* (2018). The increased CP content in this study might have been at the expense of other nutrients. Moreover, the fungal mycelium protein and the liberate cell wall bound protein probably also contributed to the increase of the soluble protein.

CONCLUSIONS

1. Seven fungi used in this study were not able to improve the rumen degradability of corn stover with low ADL content. However, *I. lacteus* produced the most beneficial result in that it can enhance the rumen degradability of the corn stover with high ADL content. Therefore, this fungus can improve efficiencies in ruminant feeding in the future.
2. This study first time indicated that not all the white rot fungi screened by SV could effectively improve the rumen degradability of corn stover.
3. Longer treatment times, as well as smaller particle sizes, increased the efficiency of *I. lacteus* to improve the rumen degradability of high ADL content of corn stover.

ACKNOWLEDGEMENTS

The authors appreciate the financial support from the Ministry of Science and Technology of the People's Republic of China (2015DFG32360).

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Article submitted: October 22, 2018; Peer review completed: November 26, 2018; Revisions accepted: December 7, 2018; Published: December 11, 2018.
DOI: 10.15376/biores.14.1.895-907