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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Treatment of Corn Straw with *Pleurotus ostreatus*, *Pleurotus eryngii* and *Lentinula edodes* to Improve the Digestibility of the Lignocellulosic Complex [*]

Aydan ATALAR[1*] **Nurcan CETİNKAYA[2]**

[1*]*Çaycuma Food and Agriculture Vocational School, Zonguldak Bulent Ecevit University, Zonguldak, Turkey*
[2]*Department of Animal Nutrition and Nutritional Diseases. Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*

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*: https://orcid.org/0000-0001-5103-7047
: https://orcid.org/0000-0002-9977-2937

***Corresponding author's:**
Aydan ATALAR
Çaycuma Food and Agriculture Vocational School, Zonguldak Bulent Ecevit University, Zonguldak, Turkey.
✉: aydan.atalar@beun.edu.tr
Mobile telephone : +90 (546) 873 42 24
Telephone : +90 (372) 261 33 79
Fax : +90 (372) 261 33 81

Abstract: The objectives of the present study were to investigate the degradation of lignocellulosic complex of corn straw (CS) by the treatment with *Pleurotus ostreatus* (PO), *Pleurotus eryngii* (PE) and *Lentinula edodes* (LE) and to determine both the most effective fungus and incubation time. The chopped corn straws were treated with PO, PE and LE, and incubated for 10, 20, 30 and 40 days at 26 ^{0}C. Chemical composition of control (CS-C) and treated corn straw (CS-PO, CS-PE and CS-LE) samples were determined. The *in vitro* true digestibilities of dry matter (IVTDMD), neutral detergent fiber (IVTNDFD), acid detergent fiber (IVTADFD) and acid detergent lignin (IVTADLD) of CS-C, CS-PO, CS-PE and CS-LE were determined by Ankom Daisy^{II} incubator. PO treatment at 30 days incubation of corn straw increased the *in vitro* true digestibility by approximately 17%. The obtained results showed that the most effective fungus was *Pleurotus ostreatus* and incubation time was 30 days.

Keywords: Corn straw, digestibility, *Lentinula edodes*, *Pleurotus eryngii*, *Pleurotus ostreatus*.

Mısır Samanının *Pleurotus ostreatus*, *Pleurotus eryngii* ve *Lentinula edodes* ile Muamele Edilerek Lignoselülozik Kompleksin Sindirilebilirliğinin Artırılması

***Sorumlu yazarın:**
Aydan ATALAR
Zonguldak Bülent Ecevit Üniversitesi, Çaycuma Gıda ve Tarım Meslek Yüksekokulu, Zonguldak, Türkiye.
✉: aydan.atalar@beun.edu.tr
Cep telefonu : +90 (546) 873 42 24
Telefon : +90 (372) 261 33 79
Faks : +90 (372) 261 33 81

Öz: Bu çalışmada mısır samanının *Pleurotus ostreatus* (PO), *Pleurotus eryingii* (PE) ve *Lentinula edodes* (LE) ile muamele edilip lignoselülozik kompleksin parçalanması ve en etkin mantar türü ile inkübasyon zamanının belirlenmesi amaçlandı. Mısır samanı PO, PE ve LE miselleri ile muamele edilip inkübatörde 26 ^{0}C'da 10, 20, 30 ve 40 günlük inkübasyonlara bırakıldı. Her bir inkübasyondan sonra in vitro gerçek kuru madde sindirilebilirliği (IVGKMS), *in vitro* gerçek organik madde sindirilebilirliği (IVGOMS) *in vitro* gerçek nötral deterjan fiber sindirilebilirliği (IVGNDFS), *in vitro* gerçek asit deterjan fiber sindirilebilirliği (IVGADFS), *in vitro* gerçek asit deterjan lignin sindirilebilirliği (IVGADLS) ANCOM DAİSY inkübatör tekniği ile belirlendi. Ortalama OM, KM, NDF, ADF, ADL'nin IVGS değerleri PO, PE ve LE muamelelerinin inkübasyon süreleri artışına parelel olarak yükseldi. Mısır samanının *Pleurotus ostreatus* ile 30 gün muamele edilmesi sonucunda mısır samanının *in vitro* gerçek sindirilebilirliğinin yaklaşık %17 arttığı belirlendi. Bulunan sonuçlar en etkin mantar türünün *Pleurotus ostreatus*, inkübasyon süresinin de 30 gün olduğunu gösterdi.

Anahtar kelimeler: Mısır samanı, sindirilebilirlik, *Lentinula edodes*, *Pleurotus eryngii*, *Pleurotus ostreatus.*

[*] *This study was produced from the Ph. Doctoral thesis.*

INTRODUCTION

In Turkey, corn agriculture is carried out in approximately 60 provinces and production is supported by the government so that it increased to 5.6 million tons in 2018. The yield of corn straw was around 5.6 million tons in 2018 (TMMOB, 2018).

The straws are rich in crude fiber due to the containing of plant stems and leaves even stover thus the nutritional value is very low. Microbial enzymes produced by digestive enzymes in ruminants and rumen microorganisms are not effective in lignin digestion. Non-digestible lignin reduces both the digestibility and utilization of feeds (Naser et al., 2011). For centuries, research has been conducted to break down the lignocellulosic complex found in cell walls of straws and to increase the availability of cell wall elements such as cellulose and hemicellulose. Biological (enzyme, white rot fungi and bacteria treatment) methods, especially, white rot fungi can depolymerize and mineralize lignin with high molecular weight through ligninolytic enzymes (Han, 2001). The digestibility of straws depends on the depolymerization of structural carbohydrates. With enzymatic degradation of macromolecules, the digestibility of straw is increased (Fazaeli et al., 2004). Lignocellulolytic enzymes that can be isolated from fungiform peroxidases and oxidases, while hydrolytic enzymes produce cellulase, hemicellulase, pectinase, chitinase, amylase, protease, esterase, and mannase (Godliving, 2012). Delignification can change according to different fungi species (Akinfemi et al., 2010). White rot fungi attack the lignin polymer and break lignol bonds and aromatic rings hence digestibility of crop residues is increased.

Formerly, studies on the improvement of digestibility of crop residues by the treatment of lignin digesting white rod fungi have been increased. In one of the reported studies, fermentation of palm stem with *P. ostreatus* for 3 months had significantly ($p<0.05$) decreased the contents of NDF, ADF, hemicellulose and lignin of palm stem. Fermentation of animal feed ingredients with *P. ostreatus* to improve the quality was suggested, especially feed used for ruminants (Metri & Warl, 2018).

Corn stover was pretreated with *Trametes versicolor, Pleurotus ostreatus, Phanerochaete chrysosporium, Coriolopsis gallica, Pleurotus sajor-caju* and *Lentinula edodes* to investigate the potential fungus among six fungi to enhance the production of products such as enzymes, sugars, and ethanol. The most effective fungus with the highest lignin degradation was determined as 38.29% at 30 days for *P. sajor-caju* pretreatment (Ding et al., 2019).

Ceriporiopsis subvermispora was used to ferment corn stover for increasing the degradability to produce biomethane. Corn stover was treated with *C. subvermispora* for 5–90 days then anaerobically digested. It was indicated that the improvement resulted from the degradation of lignocellulosic structures and bonds. *C. subvermispora* had shown a high relative selectivity for lignin degradation. The structure of the lignin and the bonds among lignin and hemicellulose and cellulose were broken by acetyl group removal, and the enzymatic hydrolysis of cellulose was increased by 35.61%. The *C. subvermispora* modification was one of the effective methods for enhancing biomethane yield from corn stover (Huang et al., 2019).

The selectivity of the three fungal species (*I. lacteus*, *P. ostreatus* and *P. chrysosporium*) for the cellulose, hemicellulose, and lignin degradation of wheat straw was studied. The results were shown that the straw treated with *P.ostreatus* had the highest ($P < 0.001$) lignin to cellulose loss ratio. However, the loss ratio of lignin to DM of the straw treated with *I. lacteus* and *P. ostreatus* was at the same level ($P = 0.897$), which was significantly greater ($P < 0.001$) than that of the straw treated by *P. chrysosporium* (Niu et al., 2018).

Lignocellulolytic and hydrolytic enzymes prepared as feed additives are available however commercial application is not practical and cost effective. The objectives of the present study were to increase the digestibility of lignocellulosic complex of corn straw by the treatment of edible white rot fungi mainly PO, PE and LE and to determine both the most effective fungus and incubation time to prepare fermented corn straw for ruminant nutrition.

MATERIAL AND METHODS

Chemical analyses and *in vitro* digestibility experiments of treated and untreated corn straw samples were carried out in the Ruminant Feed Evaluation Laboratory of Department of Animal Nutrition and Nutritional Diseases, Ondokuz Mayıs University (OMU) Faculty of Veterinary Medicine, Samsun, Turkey. White rot fungi treatments were conducted in the Fungus Laboratory of Department of Horticulture, OMU Faculty of Agriculture, Samsun, Turkey.

Animals and rumen fluid: Florya Farm raises beef cattle for their commercial meat production purpose in Samsun, Turkey. Rumen fluid was obtained from 3 beef cattle (400-500 kg LW and 2-3 years old) which were kept in the Florya Farm. Animals were fed *ad libitum* with compound feed composed of crushed corn, barley, wheat bran and vetch seed, and corn straw. Rumen fluid was collected from the rumen of slaughtered animals into the

thermos flasks and transported immediately to the Ruminant Feed Evaluation Laboratory of Department of Animal Nutrition and Nutritional Diseases, OMU Faculty of Veterinary Medicine. Rumen fluid was strained through four layers of cheesecloth and held at 39 °C under CO_2 till *in vitro* digestibility experiments.

Feed material: Corn straw was collected from 10 farmers in Doğanca, Bafra, Samsun, Turkey. Collected corn straws were homogenously mixed and chopped into 2-3 cm in length by garden scissors. Chopped corn straws were treated with PO, PE and LE fungi mycelium.

Biological treatment method by white rot fungi: Biological treatment (preparation of corn straws, sterilization, fungi mycelium inoculation and incubation procedures) were carried out according to published methods (Stamest, 1993; Dadayli, 2014). The chopped corn straws were moistened approximately 23% with water and then mixed thoroughly by hand. Then 100 g sample was weighed and transferred into the flasks and holes were opened in the center with the pens for the insertion of the fungi mycelium. Then after flasks were covered with two layers of aluminum foil to prevent contact with water and air. The prepared samples were sterilized in an autoclave at 1 atm pressure for 1 hour at 121 °C. The treated corn straws with PO, PE and LE fungi mycelium, incubated for 10, 20, 30 and 40 days in an incubator at 26°C. After each 10 days, the treated corn straws were dried at 65 °C for 24 hours. The dried white rot fungus treated samples were passed through a mill with a diameter of 1 mm for chemical analysis and in vitro digestibility experiments.

***Chemical analysis*:** Dry matter (DM), crude protein (CP), ether extract (EE) and crude ash (CA) of all samples were analyzed according to AOAC methods (AOAC 2006). Organic matter (OM) was estimated by using the DM and CA values of samples. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents of samples were determined with the method described by Van Soest et al. (1991) using the ANKOM 200 Fiber Analyzer. Metabolic energy (ME) values were calculated by using of ADF % values of samples with the following equation (Kirchgessner & Kellener, 1981). ME (MJ/kg KM) =14.70-0.150 x ADF

***Preparation of bags for Ankom Daisy^{II} in vitro fermentation system*:** The bags (Ankom F57 filter bag, Ankom Technology Corp., Fairport, NY, USA) were rinsed in acetone for 3 min. Then they were dried at room temperature. The dried bags were dried in an oven at 60 °C for 8 h. and they were weighed. The bags containing biologically treated corn straw samples were weighed (0.50 ± 0.01 g per bag, triplicate bags per treatment). The bags were sealed by an impulse bag sealer (Ankom 1915/1920 Heat Sealer, Ankom Technology Corp., Fairport, NY, USA). The bags without substrate were also included as a blank.

***Ankom Daisy^{II} incubation technique*:** The Ankom Daisy^{II} in vitro fermentation system (Ankom Technology Corp. Fairport, NY, USA) was used for the determination of in vitro true digestibility of fungus treated corn straw samples. The used procedure is based on the method described by Czerkawski and Breckenridge, (1977). Buffer solutions were prepared as described by the Ankom Daisy^{II} in vitro fermentation system procedure (ANKOM, 2020). The prepared bags were placed into jars; 1600 ml buffer solution and 400 ml rumen fluid were poured into each digestion jar. After 48 h of incubation, bags were rinsed under running tap water and dried. NDF, ADF and ADL contents of samples were analyzed (Van Soest et al., 1991). The bags were then dried at 105 °C for 12 hours then burned at 550 °C for 4-6 hours. *In vitro* true NDF, ADF and ADL digestibilities based on DM and OM samples were estimated by using equations described in Ankom Technology Method 3 (ANKOM, 2020).

***Statistical analysis*:** White rod fungi (i=1,…,3) and four incubation times (j=1,…,4) were modeled with factorial design as following; $y_{ijk} = \mu + a_i + (ab)_{ij} + e_{ijk}$ where, y_{ijk}: observation for i. fungus, μ: population or overall means, a_i: effect of i. treatment group, $(ab)_{ij}$: interaction effect of i. treatment group and j. incubation time, e_{ijk}: individual error terms. The least square equation was executed by the GLM procedure (1978). Data were summarized with deterministic statistics with means and their standard error of means. Differences among means were determined by Duncan's multiple range tests, and also differences for interaction effects were tested with Tukey's range test (SAS, 2007).

RESULTS

The chemical compositions of samples are shown in Table 1.

The *in vitro* true NDF, ADF and ADL digestibility values of the treated corn straw with PO are given in Table 2. Differences were found significant between $IVTNDFD_{DM}$ and $IVTNDFD_{OM}$ values of samples ($P<0.01$). The $IVTADLD_{DM}$ and $IVTADLD_{OM}$ values of the samples were not different between at 30 and 40 days of incubation but the difference between the other incubations were significant ($P<0.01$).

The true *in vitro* NDF, ADF and ADL digestibility values of PE treated corn straw samples are shown in Table 3. The differences between mean $IVTD_{DM}$ and $IVTD_{OM}$ values of all samples for NDF and ADF contents were significant ($P<0.01$). However, $IVTADLD_{DM}$ and $IVTADLD_{OM}$ values of the samples were not significant for 20 and 30 days incubations.

Table 1. Chemical composition of 10, 20, 30 and 40 days incubation of corn straw treated with *Pleurotus ostreatus, Pleurotus eryngii* and *Lentinula edudes* (mean ± SE).

CN %	CS-C 0 d	CS-PO 10 d	CS-PO 20 d	CS-PO 30 d	CS-PO 40 d	CS-PE 10d	CS-PE 20d	CS-PE 30d	CS-PE 40d	CE-LE 10d	CS-LE 20d	CS-LE 30d	CS-LE 40d
DM	94.4±0.68	94.3±0.75	93.8±0.53	93.3±0.55	92.8±0.87	93.7±0.81	93.3±0.76	92.4±0.85	92.1±0.69	93.0±0.71	92.7±0.69	92.5±0.85	92.1±0.65
CA	6.80±0.35	7.35±0.43	7.20±0.39	7.01±0.49	6.70±0.85	6.90±0.76	6.80±0.37	6.20±0.88	6.10±0.91	6.51±0.67	6.50±0.90	6.32±0.33	6.20±0.48
OM	87.6±0.75	86.9±0.63	86.6±0.58	86.29±0.89	86.1±0.65	86.8±0.47	86.5±0.56	86.2±0.45	86.0±0.69	86.5±0.56	86.2±0.79	86.1±0.68	85.9±0.74
CP	5.89±0.23^{a}	7.50±0.31^{a}	9.0±0.43^{b}	10.0±0.54^{C}	10.1±0.63^{C}	7.68±0.47^{a}	7.95±0.29^{b}	8.0±0.32^{b}	8.80±0.54^{b}	7.71±0.67^{a}	7.98±0.65^{b}	8.26±0.45^{b}	8.78±0.53^{b}
EE	1.17±0.03	1.22±0.02	1.25±0.06	1.24±0.03	1.22±0.01	1.18±0.02	1.17±0.03	1.18±0.05	1.16±0.01	1.17±0.04	1.21±0.05	1.16±0.03	1.19±0.07
NDF	75.6±0.67^{a}	70.0±58^{a}	66.4±0.69^{a}	65.5±0.55^{b}	60.8±0.71^{b}	73.4±0.52^{b}	67.6±0.64^{a}	67.0±0.41^{b}	66.8±0.18^{b}	74.4±0.63^{a}	69.3±0.74^{b}	67.8±0.63^{b}	66.7±0.48^{b}
ADF	40.2±0.17^{a}	38.6±0.21^{a}	36.7±0.15^{b}	34.7±0.34^{b}	34.4±0.45^{b}	37.6±0.32^{b}	35.8±0.28^{a}	34.6±0.31^{b}	34.2±0.26^{b}	39.8±0.43^{a}	39.7±0.29^{a}	38.8±0.33^{b}	38.4±0.30^{b}
ADL	9.90±0.11^{a}	7.5±0.10^{b}	6.50±0.11^{c}	6.4±0.12^{c}	5.77±0.13^{c}	8.15±0.15^{a}	8.01±0.15^{a}	7.10±0.14^{b}	4.62±0.10^{c}	8.81±0.13^{a}	8.21±0.12^{a}	7.11±0.11^{b}	6.51±0.10^{c}
ME_{ADF}	9.30±0.10^{a}	9.57±0.12^{a}	9.82±0.13^{b}	10.0±0.14^{b}	10.0±0.15^{b}	9.70±0.13^{a}	9.90±0.12^{a}	10.1±0.16^{b}	10.1±0.11^{b}	9.40±0.12^{a}	9.41±0.15^{a}	9.70±0.18^{b}	9.80±0.17^{b}

^{a,b,c} Differences between the means with different superscripts on the same row were significant (P<0.01, n = 10). d: day, DM: Dry matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, CA: Crude Ash, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin, ME: Metabolic Energy, CN: Crude Nutrient, CS-C: Corn Straw-Control, CS-PO: *Pleurotus ostreatus* treated corn straw, CS-PE: *Pleurotus eryngii* treated corn straw,CS-LE: *Lentinula edodes* treated corn straw.

Table 2. *In vitro* true NDF, ADF, and ADL digestibility values based on DM and OM for 10, 20, 30, and 40 days of incubation of corn straw treated with *Pleurotus ostreatus*.

***In vitro* True Digestibility Values (%)**	**CS-C**	**CS-PO_{10}**	**CS-PO_{20}**	**CS-PO_{30}**	**CS-PO_{40}**
$IVTNDFD_{DM}$	60.73±2.54^{e}	64.89±1.99^{d}	69.84±1.95^{c}	70.76±2.31^{b}	74.64±2.13^{a}
$IVTNDFD_{OM}$	65.31±2.09^{e}	68.58±1.95^{d}	73.80±2.29^{c}	74.78±1.97^{b}	78.88±1.32^{a}
$IVTADFD_{DM}$	35.63±1.12^{d}	36.48±1.45^{c}	36.74±1.11^{c}	38.84±1.35^{b}	40.87±1.54^{a}
$IVTADFD_{OM}$	36.05±1.00^{c}	38.55±1.12^{c}	38.83±1.41^{c}	41.05±1.17^{b}	43.2 ±1.21^{a}
$IVTADLD_{DM}$	-	4.47±0.16^{c}	6.33±0.07^{b}	6.37±0.98^{b}	7.40±0.45^{a}
$IVTADLD_{OM}$	-	4.24±0.18^{c}	6.69±0.01^{b}	6.73±0.09^{b}	7.82±0.23^{a}

^{a,b,c,d,e} Differences between the means with different superscripts on the same row were significant (P<0.01, n=10). CS-C: Corn straw control, CS-PO: corn straw treated with *Pleurotus ostreatus*, IVTNDFD: *in vitro* true NDF digestibility, IVTADFD: *in vitro* true ADF digestibility, IVTADLD: *in vitro* true ADL digestibility.

Table 3. *In vitro* true NDF, ADF, and ADL digestibility values based on DM and OM at 0, 10, 20, 30, and 40 days of incubation of corn straw treated with *Pleurotus eryngii*.

***In vitro* True Digestibility Values (%)**	**CS-C x̄ ± SE**	**CS-PE_{10} x̄ ± SE**	**CS-PE_{20} x̄ ± SE**	**CS-PE_{30} x̄ ± SE**	**CS-PE_{40} x̄ ± SE**
$IVTNDFD_{DM}$	60.73±2.54^{d}	71.29±1.89^{c}	71.54±1.93^{c}	72.27±2.14^{b}	78.43±1.89^{a}
$IVTNDFD_{OM}$	65.31±2.09^{d}	75.34±2.04^{c}	75.61±1.99^{c}	76.30±2.09^{b}	82.89±1.71^{a}
$IVTADFD_{DM}$	35.63±1.12^{c}	36.24±1.54^{b}	36.62±1.09^{b}	38.02±1.13^{b}	39.89±1.36^{a}
$IVTADF_{OM}$	36.05±1.00^{c}	38.30±1.15^{b}	38.70±1.09^{b}	38.18±1.43^{b}	45.57±1.54^{a}
$IVTADLD_{DM}$	-	4.24 ± 0.03^{c}	7.07 ± 0.05^{b}	8.13 ± 0.13^{a}	8.21 ± 0.05^{a}
$IVTADLD_{OM}$	-	4.47 ± 0.09^{c}	7.47 ± 0.12^{b}	8.59 ± 0.04^{a}	8.68 ± 0.11^{a}

^{a,b,c,d} Differences between the means with different superscripts on the same row were significant (P<0.01, n = 10).CS-C: Corn straw control, CS-PE: corn straw treated with *Pleurotus eryngii*, IVTNDFS: *in vitro* true NDF digestibility, IVTADFD: *in vitro* true ADF digestibility, IVTADLD: *in vitro* true ADL digestibility.

The *in vitro* true NDF, ADF and ADL digestibility of corn straw treated with LE are shown in Table 4. Significant differences were found between $IVTNDFD_{DM}$ and $IVTNDFD_{OM}$ values of samples (P<0.01). The mean values of $IVTADFD_{DM}$ and $IVTADFD_{OM}$ of samples were not significant at 10 and 20; 30 and 40 days of incubation periods but the differences among the others were significant (P<0.01). The mean $IVTADLD_{DM}$ and $IVTADLD_{OM}$ values of the same samples were not significant between 20 and 30 days of incubation, but the differences between the other incubation times were significant (P<0.01).

Table 4. *In vitro* true NDF, ADF, and ADL digestibility values based on DM and OM at 0, 10, 20, 30, and 40 days of incubation of corn straw treated with *Lentinula edodes.*

***In vitro* True Digestibility Values (%)**	**CS-C x̄ ± SE**	**CS-LE_{10} x̄ ± SE**	**CS-LE_{20} x̄ ± SE**	**CS-LE_{30} x̄ ± SE**	**CS-LE_{40} x̄ ± SE**
$IVTNDFD_{DM}$	60.73±2.54^{e}	71.12±1.56^{d}	72.20±1.90^{c}	73.95±2.17^{b}	79.27±1.99^{a}
$IVTNDFD_{OM}$	65.31±2.09^{e}	75.16±2.27^{d}	76.30±1.87^{c}	78.16±1.65^{b}	83.78±1.32^{a}
$IVTADFD_{DM}$	35.63±1.12^{c}	40.79±1.15^{b}	40.99±1.21^{b}	42.07±1.41^{a}	42.31±1.32^{a}
$IVTADFD_{OM}$	38.05±1.00^{c}	43.11±1.37^{b}	43.32±1.11^{b}	44.46±1.09^{a}	44.71±1.28^{a}
$IVTADLD_{DM}$	-	6.82±0.05^{c}	7.96±0.16^{b}	8.22±0.09^{b}	94.14±0.08^{a}
$IVTADLD_{OM}$	-	6.49±0.08^{c}	8.41 ± 0.12^{b}	8.69 ± 0.15^{b}	9.23 ± 0.06^{a}

^{a,b,c,d} Differences between the means with different superscripts on the same row were significant (P<0.01, n = 10). CS-C: Corn straw control, CS-PO: corn straw treated with *Pleurotus ostreatus*, CS-PE: corn straw treated with *Pleurotus eryngii*, CS-LE: corn straw treated with *Lentinula edodes*. IVTNDFD: *in vitro* true NDF digestibility, IVTADFD *in vitro* true ADF digestibility, IVTADLD: *in vitro* true ADL digestibility

The *in vitro* true DM digestibility values of corn straw treated with PO, PE and LE for are shown in Table 5. The differences between the incubation times of the $IVTD_{DM}$ values of samples were statistically significant (P<0.05). The differences between the mean values of the PO, PE and LE at the same incubation were significant (P<0.05).

Table 5. *In vitro* true DM digestibility values at 0, 10, 20, 30, and 40 days of incubation of corn straw treated with *Pleurotus ostreatus, Pleurotus eryngii* and *Lentinula edodes*.

IVTDMD values based on incubation times (%)	**CS-C x̄ ± SE**	**CS-PO x̄ ± SE**	**CS-PE x̄ ± SE**	**CS-LE x̄ ± SE**
$IVTDMD_{10}$	59.45 ± 1.11^{b}	60.51 ± 0.98^{a}	60.82±1.00^{a}	60.18±1.41^{a}
$IVTDMD_{20}$	59.45 ± 2.29^{d}	70.55 ± 1.98^{c}	67.18±1.34^{b}	66.96±1.77^{a}
$IVTDMD_{30}$	59.45 ± 1.56^{d}	74.90 ± 1.19^{c}	69.18±1.63^{a}	69.28±1.54^{a}
$IVTDMD_{40}$	59.45 ± 2.03^{d}	76.50 ± 1.99^{c}	71.24±1.88^{b}	73.04±1.92^{a}

^{a,b,c,d} Differences between the means with different superscripts on the same row were significant (P<0.05, n = 10). CS-C: Corn straw control, CS-PO: corn straw treated with *Pleurotus ostreatus*, CS-PE: corn straw treated with *Pleurotus eryngii*, CS-LE: corn straw treated with *Lentinula edodes*. IVTDMD: *in vitro* true dry matter digestibility.

DISCUSSION

Chemical composition of untreated corn stover was found similar to previously published values for corn straw (Russel, 1986; Alhassan & Aliyu, 1991). The mean DM%, CA%, EE% and OM % values of CS-C, CS-PO_{10-40}, CS-PE_{10-40}; CS-LE_{10-40} were changed between 92.10-94.40%; 6.10-7.35%; 1.16-1.25% and 87.60-85.90% respectively.

Obtained results for DM%, CA%, EE% and OM % values were different from the reported similar study with bamboo and corn straw (Adenipekun & Okunlade, 2012). The reason for finding different results in our study may be due to different treatment methods and the 90-day long incubation period used in their study.

PO, PE and LE treatment of corn straw increased the CP% values in the present study at increasing incubation times. Akinfemi et al. (2010) reported that sorghum stover treatment with two different white rot fungi also improved digestibility and increased CP level. Langar et al. (1980) reported that wheat straw treated with *Agaricusbisporus* fungus and incubated for 26-30 days and treated with *Volvariella diplasia* and incubated for 28-30 days showed that soluble cell wall components, lignin and hemicellulose were decreased but CP content was increased. Obtained results for CP, NDF, ADF and ADL of treated corn straw samples were in line with the reported values by Langar et al. (1980). The NDF values of CS-PO

were found less than CS-PE and CS-LE at 30 and 40 days incubations. The lowest NDF value for CS-PO was 60.8% at 40 days incubation. Comparison of 10, 20, 30 and 40 days of incubations on NDF levels showed that mean values were decreased with increasing incubation times. The mean NDF values for 30 and 40 days were 65.5% and 60.8 % respectively. The results indicate that white rot fungi were degraded the lignin content of CS. Similar to our study, Bribiesca et al. (2010) reported that NDF levels of corn straw were also decreased with PO treatment for 15 days incubation. It was also shown that the digestibility of OM increased from 200 g/ mL to 309 g/mL after 49 days of incubation of wheat straw with *Ceriporiopsis subvermispora* fungus (Bribiesca et al., 2010).

Tuyen et al. (2012) reported that treated maize stover with the white rot fungi *Ceriporiopsis subvermispora*, *Lentinula edodes*, *Pleurotus eryngii*, or *Pleurotus ostreatus* at 24 °C for 0-6 weeks, showed a linear relationship ($P<0.05$) between the proportion of lignin in the original substrate for C. *subvermispora* and L. *edodes* treatments ($R^2=0.92$ and 0.96, respectively). In our study, IVTNDFD, IVTADFD, IVTADLD and IVTDMD of CS-C, CS-PO, CS-PE and CS-LE were increased with increasing incubation times for 0-40 days (Tables 4-7). White rot fungus mainly *Pleurotus ostreatus*, *Pleurotus eryngii* and *Lentinula edodes* treatments had a very good effect to improve the digestibility of corn straw as IVTNDFD reported values by Tuyen et al. (2012). Among the estimated values of IVTNDFD, IVTADFD and IVTADLD of fungi treated corn straw between 0-40 days incubations as shown in Tables 6 and 7, the most effective fungus was PO and incubation times were 30 and 40 days. Even though the estimated digestibility of corn straw for 40 days incubated PO was similar to 30 days incubation with the same fungus, unwanted physical appearances occurred after 40 days of incubation time. In the previously reported paper, *in situ* degradability of NDF after PO treatment of corn stover was also increased (Díaz-Godínez & Sánchez 2002). Biologically treated roughages had higher digestibility for most of the nutrients (both cell walls and cell soluble) with an increase in crude protein content as compared to untreated material, besides ensuring more fermentable substrates in the rumen (Mahesh & Mohini, 2013). In a reported paper, exogenous fibrinolytic enzyme supplementation to the high fiber containing diet enhanced the feed intake and improved growth performance in Lezhi black goats (Song et al., 2018). Findings of our study also showed that all three fungi PO, PE and LE treatments of corn straw increased the true digestibility of NDF, ADF, ADL and DM at all incubation times between 0-40 days (Tables 4-7). This is a very good indication that treatment of corn straw with PO, PE and LE can break the lignocellulose complexes and enhance its energy and protein values for ruminants.

Raghuwanshi et al. (2014) reported that wheat straw treatment with the tannase enzymes isolated from *Ganoderma spp.* increased CP by 1.28% and degraded lignin, thus the digestibility of wheat straw was increased (Kundu et al. 2005). Some of fungi species have high ligninolytic activity, *Pleurotus spp.* is one of the type of fungi improves digestibility of crop residues. Bribiesca et al. (2010) demonstrated that corn straw treated with *P. ostreatus* for 15 days increased the crude protein, soluble protein, soluble carbohydrates, ash and decreased neutral detergent fiber. In a study, Cyathusstercoreus was the best observed among four white rot fungi (*Phonerochaete chrysosporium*, *Auricularia polytricha*, and *Sporotrichum pulverulentum*) tested with maximal ligninolytic and minimal cellulolytic and hemicellulosic activity leading to minimizing nutritional loss (Bakshi et al., 2011).

The ruminal degradability of various varieties of wheat straw treated with *Ceriporiopsis subvermispora* and *Lentinula edodes* were shown a very good degradation of lignin on a more mature straw (89.0%) in comparison with the straw harvested at an earlier stage (70.7%) (Nayan et al., 2019).

Shrivastava et al. (2011) reported a significant reduction in the number of cell wall components such as ADF, NDF, hemicelluloses, lignin and cellulose by 35.00, 39.88, 45.00, 37.48 and 37.86% in *P. ostreatus* fermented straw; 30.04, 33.85, 39.90, 31.29 and 34% in *T. versicolor* fermented straw respectively. However, maximum efficacy was observed in terms of low carbohydrate consumption per unit of lignin degradation for *P. ostreatus* on day 10 (17.12%) compared with *T. versicolor* (16.91%) on day 30. Hassim et al. (2012) reported an increase in rumen degradation of oil palm fronds leaves for white rot fungi *Ceriporiopsis subvermispora* (13%) and *Lentinula edodes* (10%) treatments but not for supplementation with yeast or enzymes. White rot basidiomycetes are the most potent lignin degraders of all known microorganisms (Galhaup et al., 2002). Adenipekun et al. (2012) reported that the degradation of rattan wood and maize stovers were increased with *Pleurotus ostreatus* treatment, and the crude protein in the treated substrates for both rattan wood and maize stovers was higher than the untreated substrates throughout the period of incubation (Adenipekun and Okunlade, 2012). On the other hand, Belewu and Belewu, (2005) indicated that the addition of fungal protein to rice husk increased degradability and crude protein content of rice husk. Jonathan et al. (2004) also reported an increase in fungal protein when maize stovers were treated with *Pleurotus tuber-regium*. Thi Huyen et al. (2019) reported that biological treatments methods by using white rot fungi were one of the best alternatives to improve the nutritional value of rice straw since the effects of the utilization of

fungal treated rice straw in the diet of sheep on feed intake and nutrient digestibility was better than untreated or urea treated rice straw.

CONCLUSIONS

The obtained results show that the most effective incubation time is 30 days and white rot fungus is *Pleurotus ostreatus*. The lignocellulosic complex digestibility of corn straw was increased by 17 % at 30 days incubation with *Pleurotus ostreatus*. Besides, the estimated digestibility of corn straw for 40 days incubated corn straw with *Pleurotus ostreatus* is also close to 30 days incubation with the same fungus, however, odor and color changes occurred after 40 days of incubation time. Feeding trials may be required to observe the effects of fermented corn straw on animal productivity before putting into commercial scale application of fermented corn straw preparation. As a result, the present study indicated that all studied white rot fungi *Pleurotus ostreatus, Pleurotus eryngii* and *Lentinula edodes* have a potential to improve the quality of corn straw for ruminant nutrition.

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REFERENCES

Adenipekun, C.O. & Okunlade, O.A. (2012). Biodegradation of rattan wood and maize stovers by *Pleurotus ostreatus. Journal of Natural Sciences*, ***10***, 49-57.

Akinfemi. A., Adu, O.A. & Doherty, F. (2010). Conversion of sorghum stover into animal feed with white-rot fungi: *Pleurotus ostreatus* and *Pleurotus pulmonarius. African Journal of Biotechnology*, ***9***, 1706–1712.

Alhassan, W.S. & Aliyu, S.U. (1991). Studies on urea ammonia treatment of maize straw: treatment method and potential for dry season feeding of cattle in Northern Nigeria. *Animal Feed Science and Technology*, ***33***, 289-295.

ANKOM. (2020). Ankom Technology Method 3. *In vitro* True Digestibility using the DAISY^{II} Incubator. https://www.ankom.com/sites/default/files/document-files/Method_3_Invitro_D200_D200I.pdf. (2 Feb 2020).

AOAC. (2006). *The official methods of analysis of AOAC international, 18th edn.* Association of Official Analytical Chemists. Arlington, USA (2), 59-62.

Bakshi, M.P.S., Wadhwa, M., Gangwar, M. & Lamba, J.S. (2011). Biodegradation of wheat straw by lignolytic fungi. *Indian Journal of Animal Nutrition*, ***28***, 371-376.

Belewu, M.A. & Belewu, K.Y. (2005). Cultivation of Mushrooms (*Volvariella volvacea*) on banana Leaves. *African Journal of Biotechnology*, ***4***, 1401-1403.

Bribiesca, J.E.R., Sanchiez, S.A., Hernandez, L.M., Chavira, J.S., Galaviz, Monterrosa, R.G.C. & Lopez, V.S. (2010). Influence of *Pleurotus ostreatus* spent corn straw on performance and carcass characteristics of feedlot Pelibuey lambs. *Indian Journal of Animal Science*, ***80***, 754-757.

Czerkawski, J.W. & Breckenridge, G. (1977). Design and development of long-term rumen simulation technique (RUSITEC). *British Journal of Nutrition*, ***38***, 271-384.

Dadayli, G., (2014). *The effects of fragmentation and casing on biological efficiency and yield of Pleurotus eryngii mushroom in substrates prepared by tea waste.* OMU Institute of Science Sciences. Samsun, Turkey.

Díaz-Godínez, G. & Sánchez, C. (2012). *In situ* digestibility and nutritive value of maize straw generated after *Pleurotus ostreatus* cultivation. *Canadian Journal of Animal Science*, ***82***, 617-619.

Ding, C., Wang, X. & Li, M. (2019). Evaluation of six white-rot fungal pretreatments on corn stover for the production of cellulolytic and ligninolytic enzymes, reducing sugars, and ethanol. *Applied Microbiology and Biotechnology*, ***103(14)***, 5641-5652.

Fazaeli, H., Mahmodzadeh, H., Jelan, Z.A., Rouzbenhan, Y., Liang, J.B. & Azizi, A. (2004). Utilization of fungal treated wheat straw in the diet of late lactating cow. *Asian-Australasian Journal of Animal Sciences*, ***17***, 467-472.

Godliving, Y.S.M. (2012). Lignocellulolytic enzymes from tropical fungi: Types, substrates and applications. *Scientific Research and Essays*, ***7***, 1544-1555.

Han, Y.W. (2001). Microbial utilization of straw (a review). *Advances in Applied Microbiology*, ***23***, 119-153.

Hassim, H.A., Lourenço, M., Goh, Y.M., Baars, J.J.P. & Fievez, V. (2012). Rumen degradation of oil palm fronds is improved through pre digestion with white rot fungi but not through supplementation with yeast or enzymes. *Canadian journal of Animal Science*, ***92***, 79-87.

Huang, W.B., Wachemo, A.C., Yuan, H.R. & Li, X.J. (2019). Modification of corn stover for improving biodegradability and anaerobic digestion

performance by Ceriporiopsis subvermispora. *Bioresource Technology*, ***283***, 76-85.

Jonathan, S.G., Fasidi, I.O. & Ajayi, E.J. (2004). Physico-Chemical studies on Volvariella esculenta (Mass) Singer, a Nigerian edible fungus. *Food Chemistry*, ***85***, 339-342.

Kirchgessner, M. & Kellener, R.J. (1981). Schatzung Des Energetischen Futterwertes von Grün und Rauhfutter Durch Die Cellulase method. *Landwirtschaftliche Forschung*, ***34***, 276-281.

Kundu, S.S., Mojumdar, A.B., Singh, K.K. & Das, M.M., (2005). Improvement of poor quality roughages. In: S.S. Kundu, S.K. Mahanta, S. Singh, P.S. Pathak (eds) *Roughage Processing Technology*, 193-209p, Satish Serial Publishing House, Delhi, India.

Langar, P.N., Sehgal, J.P. & Garcha, H.S. (1980). Chemical changes in wheat and paddy straw after fungal cultivation. *Indian Journal of Animal Science*, ***50***, 942-946.

Mahesh, M.S. & Mohini, M. (2013). Biological treatment of crop residues for ruminant feeding: A review. *African Journal of Biotechnology*, ***12***, 4221-423.

Metri, Y. & Warly, L. (2018). Suyitman. Biodegradation of lignin by white rot fungi (*Pleurotus ostreatus*) to decrease the fibre components in the palm midrib. *Pakistan Journal of Nutrition*, ***17(2)***, 71-75.

Naser, M.S., Bayaz, A.Z., Ramin, S., Alireza, A., Abolfazl, A.G. & Mohebbizadeh, M. (2011). Determining nutritive value of soybean straw for ruminants using nylon bags technique. *Pakistan Journal of Nutrition*, ***10***, 838-841.

Nayan, N., Van Erven, G., Kabel, M.A., Sonnenberg, A.S.M., Hendriks, W.H. & Cone, J.W. (2019). Improving ruminal digestibility of various wheat straw types by white-rot fungi. *Journal of the Science of Food and Agriculture*, ***99(2)***, 957-65.

Niu, D., Zuo, S., Jiang, D., Tian, P., Zheng, M. & Xu, C. (2018). Treatment using white rot fungi changed the chemical composition of wheat straw and enhanced digestion by rumen microbiota in vitro. *Animal Feed Science and Technology*, ***237***, 46-54.

Raghuwanshi, S., Misra, S. & Saxena, R. K. (2014). Treatment of wheat straw using tannase and white-rot fungus to improve feed utilization by ruminants. *Journal of Animal Science and Biotechnology*, ***5***, 13-20.

Russell, J.R. (1986). Influence of harvest date on the nutritive value and ensiling characteristics of maize stover. *Animal Feed Science and Technology*, ***14***, 11-27.

SAS. (2007). Statistical software, SAS Campus Drive. Cary.

Shrivastava, B., Thakur, S., Khasa, Y.P., Gupte, A., Puniya, A.K. & Kuhad, R.C. (2011). White rot fungal conversion of wheat straw to energy rich cattle feed. *Biodegradation*, ***22***, 823-831.

Song, S.D., Chen, G.J., Guo, C.H., Rao, K.Q., Gao, Y.H., Peng, Z.L., Zhang, Z.F., Bai, X., Wang, Y., Wang, B.X., Chen, Z.H., Fu, X.S. & Zhu, W.L. (2018). Effects of exogenous fibrolytic enzyme supplementation to diets with different NFC/NDF ratios on the growth performance, nutrient digestibility and ruminal fermentation in Chinese domesticated black goat. *Animal Feed Science and Technology*, ***236***, 170-177.

Stamest, P. (1993). *Growing gourment and medicinal mushrooms*, Ten Speed Press, Berkley.

Thi Huyen, N., Quang Tuan, B., Xuan Nghie, N., Thi Bich, T.N. & Thi Tuyet, N. (2019). Effect of using fungal treated rice straw in sheep diet on nutrients digestibility and microbial protein synthesis. *Asian Journal of Animal Science*, ***13(1)***, 1-7.

TMMOB. (2018). Turkey corn production report. http://www.zmo.org.tr/genel/bizden_detay.php?kod=30187&tipi=17&sube=0. (5 May 2020).

Tuyen, V.D., Cone, J.W., Baars, J.J.P., Sonnenberg, A.S.M. & Hendriks, W.H. (2012). Fungal strain and incubation period affect chemical composition and nutrient availability of wheat straw for rumen fermentation. *Bioresource Technology*, ***111***, 336-342.

Van Soest, P.J., Robertson, J.D. & Lewis, B.A. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, ***74***, 3583-3597.