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Determining the *In Situ* Ruminal Degradability of Some Nutrients of Wheat Straw Using Some White Rot Fungi Species

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ABSTRACT

The *Phanerochaete chrysosporium* (PC) and *Pleurotus eryng*ii (PE) were observed for their potential to increase the *in situ* dry matter (DM) and crude protein (CP) degradability of wheat straw. The wheat straw samples were inoculated with the PC and PE fungi (10% weight/volum) and incubated under solid state fermentation conditions at 30 ˚C for 30 days to determine their *in-situ* degradability and chemical composition. *In situ* ruminal degradability was determined by using four ruminal cannulated Holstein bulls (approximately 400 kg BW) fed on a diet a concentrate-to-roughage ratio of 45:55 (dry matter (DM) basis). The samples were incubated in nylon bags for 0, 4, 8, 16, 24, 48, 72 and 96 h in the rumen. The results showed that increased in situ ruminal degradation of DM and CP in PC and PE groups in comparison to the control group ($P < 0.01$). The wheat straw incubated with PC had the greatest *in situ* DM degradability after incubation for 24 h ($P < 0.01$). The inoculation of PC and PE increased *in situ* ruminal crude protein (CP) degradation ($P < 0.01$) in comparison to the other treatments. As a result, the fungal inoculants and urea treatment increased the degradability of DM and CP in wheat straw.

Siyah Alacalarda Beyaz Çürükçül Funguslar Kullanılarak Buğday Samanın *İn-Situ* Parçalanabilirliğinin Belirlenmesi

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ÖZ

Bu araştırmada, *Phanerochaete chrysosporium* (PC) ve *Pleurotus eryngii* (PE) mantar türlerinin, buğday samanının *in-situ* parçalanabilirliği sonucunda kuru madde (KM) ve ham protein (HP) değerleri üzerindeki etkileri incelenmiştir. Buğday samanı örnekleri, PC ve PE fungus türleri ile (%10 ağırlık/hacim) ile inokule edilip, *in-situ* parçalanabilirlikleri ve kimyasal bileşimleri belirlenmek üzere katı hal fermentasyon koşulları altında 30 °C'de 30 gün inkübe edilmiştir. Kanül takılı 4 baş Siyah Alaca boğa (yaklaşık 400 kg canlı ağırlığa sahip) 45:55 oranında (KM esasına dayalı) konsantre / kaba yem ile beslenmiştir. Yem örnekleri rumende naylon torbalarda 0, 4, 8, 16, 24, 48, 72 ve 96 saat süreyle inkübe edilmiştir. Sonuçlar, PC ve PE'nin inokulasyonunun, kontrol grubuna göre KM ve HP'nin *in-situ* ruminal parçalanabilirliğini yükselttiğini göstermiştir ($P < 0.01$). PC ile inkübe edilen buğday samanı, 24 saat inkübasyondan sonra en yüksek *in-situ* KM parçalanabilirliğine sahip olmuştur ($P < 0.01$). PC ve PE'nin inokulasyonu, diğer muamelelere göre *in-situ* rumen ham protein (HP) parçalanabilirliğini ($P < 0.01$) artırmıştır. Sonuç olarak, fungus inokulayonu ve üre muamelesinin, buğday samanındaki KM ve HP'in parçalanabilirliğini yükselttiği gözlemlenmiştir.

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Introduction

In the world, factors such as climatic changes, rapid urbanization and socio-economic imbalances etc narrow the production areas of quality roughage and thus decrease quality roughage production. Therefore, it occurs significant nutritional problems and considerable yield losses in livestock. Improving some alternative methods to eliminate or minimize of these losses present great importance. For this aim, it is among methods the first to mind come to utilize from some food by-products having roughage characteristic. One of these by-products is wheat straw. Wheat straw, as the most abundant agricultural by-product in Turkey, is a promising alternative type of roughage variety in ruminant feeding. Although, there are some features that limit use profit of it (Jalc et al., 1997), agricultural and food industry wastes constitute a large part of the agricultural production in the world such as 30% approximately (Gültepe and Bayram, 2019).

The major limitation of using wheat straw as a feed for ruminants low rumen degradability and low energy value (Demeyer et al., 1988; Chegeni et al., 2013) due to its high lignin content (Zadrazil and Uniya, 1995). Because, cellulose and hemicellulose are hardly degraded by the rumen microorganisms (Okano et al., 2005; Turgut, 2008). To improve the disposability of wheat straw, different physical, chemical, and biological treatments were used (Brand et al., 1991; Kalkan, 2008; Turgut, 2008). The physical methods have little effect on the digestibility of this by-product (Sundstøl and Owen, 1984; Turgut, 2008). Some chemical treatments (urea, urea plus $Ca(OH)_2$, urea plus SO_2, $Ca(OH)_2$ and NaOH plus $Ca(OH)_2$,SO_2) greatly improve the digestibility (Fahmy and Klopfenstein, 1994), but the application of these chemicals have poses also a high risk to the environment (Tuyen, 2012). Thus, biological methods that are believed to be more effective and more beneficial, have been earned importance recently. Because, biological methods are capable of degrading the lignocellulosic bonds and increasing the profitable of nutrients; thus, these methods can provide an economicaly and environmentally friendly alternative for chemical methods (Rahman et al., 2011). It was reported appling white rot fungi is quite effective biological method for delignification due to the fungi's a lot of characteristic (Reid, 1989; Eriksson et al., 1990). *Phanerochaete chrysosporium* is the model white rot fungus because of its specialized ability to degrade the abundant aromatic polymer lignin. *P. chrysosporium* releases extracellular enzymes to break up the complex three dimensional structure of lignin into components that can be utilized by its metabolism. The extracellular enzymes are nonspecific oxidizing agents (hydrogen peroxide, hydroxyl radicals) used to cleave the lignin bonds (Burdsall et al., 1974). *Pleurotus eryngii* belong to genus Pleurotus and are the largest species in oyster mushroom genus. Also white-rot fungicontaining this specie is reported to be able to secrete ligninolytic enzymes (lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase) that are associated with ligninolyric activities (Hadibarata et al., 2012).

Little information is available on the interactions between *Phanerochaete chrysosporium* and *Pleurotus eryngii* in wheat straw, especially in terms of *in situ* ruminal degradability. The objective of

our study is to investigate the effect of treatment of wheat straw with *Phanerochaete chrysosporium* and *Pleurotus eryngii* fungi species on celulose and hemicelulose degradeibility.

Material and Method

The animal material and *in situ* process

This study was conducted at the Eastern Anatolian Agricultural Research Institute in Erzurum, Turkey. The experiment process that approved by the Atatürk University Animal Experiments Local Ethics Committee, had conducted with decision and this decision procedures were followed throughout the experiment. Four head cannulated Holstein bulls that are 20-month-old (average 400 kg BW) were used 4 weeks after the cannulation surgery. It was applied two week adaptation period before the study started. The feed ingredients and the chemical composition are showed in Table 1. *In situ* rumen degradation of feed and fibre components was evaluated using the nylon bag technique (Susmel et al., 1990). The samples were incubated in nylon bags for 0, 4, 8, 16, 24, 48, 72 and 96 h in the rumen.

Preparation of feed mixtures and feeding

The mixtures were prepared in the form of combinations reported below and each mixture was tested in two repeat in each animal. I. Wheat Straw (control), (WS); II. Wheat Straw + based on dry matter %1 Urea, (WSU); III. Wheat Straw + %1Urea + fungi, Pleurotus eryngii (WSUPE); IV. Wheat Straw + %1Urea+ fungi, *Phanerochaete chrysosporium* (WSUPC); V. Wheat Straw + fungi, *Pleurotus eryngii*, (WSPE); VI. Wheat Straw + fungi, *Phanerochaete chrysosporium*, (WSPC). The cannulated animals fed in individual paddocks were fed with 55/45 roughage / concentrate in accordance with the declarations of NRC (NRC, 1985) and clean water was kept in front of the animals.

Microorganisim, chemical and fungal inoculant preparation

In this study two fungi varietes and urea were used. *Phanerochaete chrysosporium* (NRRL 6370) was obtained from the ARS Culture Collection, USDA, USA. *Pleurotus eryngii* was purchased from a commercial company and reproduced in the laboratory of Biology Department of the Faculty of Science of Atatürk University. In feed mixture groups, it was used 1% ratio from granular urea obtained by heating ammonium carbonate to 150-200 °C. The dried wheat straw was weighed to 750 g at autoclave resistant plastic bags. In order to have a moisture content of 60% was added distilled water to the straw sachets before from biological degradation. Moisture content of urea added straw samples was provided by adding dissolving in water of urea. Then the bags were closed and sterilized at 121 ^{o}C, 1 atm pressure for 15 minutes. To sterile straws prepare to solid state fermentation were inoculated solition at a rate of 10% (w / v) and achieved under aseptic conditions homogeneity. Then the straw samples were allowed to incubation in the incubator at 30 °C for 30 days.

Chemical component analysis

The DM content was measured by drying samples in an electrical oven at 105°C for 2 h (AOAC, 1990). The ash content was measured by burning the samples in a electrical furnace at 550 °C for 4 h (AOAC, 1990). Nitrogen (by Kjeldalh metod) and ether extract (by Soxhleth device) were measured according to AOAC (AOAC, 1990). Acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were measured according to the procedure recommended by Van Soest et al. (1991). The hemicellulose content was estimated by the difference between NDF and ADF, and the cellulose content was calculated based on the difference between ADF and ADL. The hemicellulose content was estimated by the difference between NDF and ADF, and the cellulose content was calculated based on the difference between ADF and ADL. The OM was measured as the difference between DM and the ash content. The component degradation ratio was calculated on the basis of the total component weight loss after pretreatment (Ma et al., 2011). Using 48 hours dry matter degradability values of roughages, metabolic energy values were determined by using the regression equation (ME, (Mj/kg DM) = 2.27563+0.1073 * KMP) developed at Rowett Research Institute (Karabulut and Canbolat, 2005).

Chemical analysis of feeds

Table 1. Chemical composition of feed ingredients used in the study

Feeds	Components (%)					
	DM	CP	CA	ADF	NDF	ME (Kcal/kg)
Concentrate	88.2	16.0	7.1	-	-	2500
Dry meadow grass	88.1	10.7	8.5	39.0	64.3	2100
Dry clover	88.7	19.1	8.5	31.0	40.0	2100
Wheat straw	91.6	3.81	7.8	49.4	73.0	1480

DM: dry matter. CP: crude protein. CA: crude ash. ADF: acid detercent fiber. NDF: Neutral detergent fiber. ME: metabolic energy

Formulations for degradability ratios

DM and CP degradability after incubation was calculated according to the following formulas (Susmel et al., 1990).

DM (degradability %)=[[((W1-W4) * DM%) – ((W3-W2) * 100)]/(W1-W4)*DM%]*100 (1)

W1=sample weight.W2=weight of empty bag in desiccator. W3=dried weight of bags taken from rumen at 70 degrees for 24 hours. W4= weight of sample evacuated bag outside 24 hours

CP (degradability %) = [(Y1-Y2)/Y1]*100 (2)

Y1= amount of crude protein before incubation. Y2= amount of crude protein after incubation

Variables of *in situ* degradation rapidit of feed samples were estimated according to the following model (Ørskov and McDonald 1979; McDonald, 1981; Susmel et al., 1990; Ribeiro, 1994).

Model D.% = a + b × [1–exp (−ct)] (3)

where D = degraded proportion at time t; a = the fraction that immediately disappears from the bag; b = N loss due to microbial activity in rumen; c = constant rate of degradation of fraction b (h−1); and t = incubation time (h).

The effective degradability (ED) was calculated using the following equation:

Effective D = a+b[bc/(c+k] (1-e-(c+k)t) (4)

where a: fast dissolution and degradation in rumen for nutrients. b: potential degradability value that requires a certain time to dissolve and degrade. c: from rumen passage rate that is assumed as 0.02/h. t is degradation time. k is flow rate from rumen.

The chemical composition components such as DM, CP, CA, NDF, ADF, ADL, OM, cellulose, hemi cellulose and the ruminal parameters such as a, b, c and k were analysed using analysis of variance (ANOVA). Between treatment and incubation time had on the *in situ* degradability parameters were analysed using the univariate tests that are part of general linear model analysis. In this process, the following mathematical model was applied.

$Yijk = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$

Where. μ: mean of the sample. ai: the effect of treatment. bj: the effect of incubation times. eijk: error. The statistical analyses were performed with the statistical software package SPSS 20.0 (SPSS. 2010).

Results

Changes in chemical composition

The chemical analysis of the feed mixtures are given in Table 2. All of the fungal treatments (except WSPC) and urea treatment reduced the DM, OM, hemicellulose, cellulose, NDF, ADF and ADL content of the wheat straw after 30 days of fermentation ($P < 0.01$). On the other hand, it was higher CP and crude fat according to the control.

The wheat straw incubated with *Pleurotus eryngii* + urea had the lowest DM, OM, NDF, ADL and hemicellulose content ($P < 0.01$), but the highest CP, cellulose and ash content ($P < 0.01$) in comparison to the other treatments. The wheat straw incubated with *Pleurotus eryngii* + urea had the lowest DM, OM, NDF, ADL and hemicellulose content ($P < 0.05$), but the highest for CP, cellulose and CA content ($P < 0.05$) in comparison to the other treatments. The DM and OM contents in the wheat straw inoculated with *Phanerochaete chrysosporium* fungi were higher than WS treatment ($P < 0.01$), were similar in point of hemicellulose and crude ash (Table 2).

Table 2. Chemical composition of wheat straw incubated with the *Phanerochaete chrysosporium*. Pleurotus eryngii Fungus. urea for 30 days (%)

Feed Mixture Groups	Variables (%) ($\overline{X} \pm S\overline{x}$)									
	DM	**OM**	**CP**	**EE**	**HC**	**ADF**	**NDF**	**ADL**	**C**	**CA**
	**	**	**	**	**	**	**	**	**	**
WSPC	92.67^{a}	85.32^{a}	10.31^{c}	3.50^{b}	27.03^{a}	33.50^{c}	60.53^{c}	7.92^{bc}	25.57^{bc}	7.34^{bc}
WSUPC	89.00^{c}	81.95^{c}	11.80^{b}	4.76^{a}	25.79^{a}	31.38^{d}	57.17^{d}	9.43^{b}	24.55^{c}	7.04^{c}
WSPE	89.63^{c}	80.60^{c}	8.86^{d}	2.58^{cd}	20.04^{b}	34.69^{c}	54.74^{e}	7.25^{c}	27.44^{b}	9.02^{a}
WSUPE	87.43^{d}	78.36^{d}	16.66^{a}	2.87^{c}	15.90^{c}	38.66^{b}	54.57^{e}	6.63^{c}	32.03^{a}	9.07^{a}
WSU	88.93^{c}	81.13^{c}	10.93^{bc}	2.19^{d}	25.51^{a}	41.98^{a}	67.49^{b}	11.31^{a}	30.66^{a}	7.80^{bc}
WS (control)	91.61^{b}	83.62^{b}	3.81^{e}	2.11^{d}	27.03^{a}	42.77^{a}	70.94^{a}	11.71^{a}	31.06^{a}	7.99^{b}
SEM	±0.29	±0.456	±0.40	±0.18	±0.86	±0.53	±0.56	±0.52	± 0.83	±0.26

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; HC: hemicellulose; ADF: acid detergent fiber; NDF: neutral detergent fiber. ADL: acid detergent lignin. C: cellulose; CA: crude ash; WSPC: wheat straw + Phanerochaete chrysosporium; WSUPC: wheat straw + urea + Phanerochaete chrysosporium; WSPE: wheat straw + Pleurotus eryngii; WSUPE: wheat straw + urea + Pleurotus eryngii; WSU: wheat straw + urea; WS: wheat straw; SEM: standard error of the mean. **: P<0.01; a-e: means in the same column with different letters differ

***In situ* degradability**

In the study *in situ* DM and crude protein degradation values (%) determined according to feed mixture groups and at different rumen incubation times are given in Table 3. Differences were found among the different treatments and incubation times for *in situ* degradation of DM; differences in the degradation of this component was observed significant ($P < 0.01$). The *Pleurotus eryngii* + wheat straw (WSPE) treatment had the highest DM degradability after 0 h and 4 h than other mixture groups. Wheat straw + *Pleurotus eryngii* (WSPE). Wheat straw + *Phanerochaete chrysosporium* (WSPC) and wheat straw + urea + *Phanerochaete chrysosporium* (WSUPC) mixture groups was found similar *in-situ* DM degradation after 8 h and 16 h, and these groups were higher than the other groups, including the control group ($P < 0.01$). In incubations after 24 hours was seen a certain degree effect superiority of PC adding mixtures. The *in situ* ruminal degradation of DM was higher in the WSPC and WSUPC mixtures than other all groups after 24 h, and a similar result was observed in the results after 72 h ($P < 0.01$). The WSUPE, WSPC and WSUPC treatments had the highest DM degradability after 48 h and 96 h.

Feed mixture groups affected CP disappearances as a function of incubation time and differences for CP disappearance between treatments groups at all rumen incubation times were observed ($P < 0.01$, table 3). The highest degradation rate of wheat straw was observed with the WSPE, which achieved a 9.61%, 17.62% respectively loss of crude protein after 0 h and 4 h incubation. In the losses of crude protein observed after 0 h. WSUPE with WSUPC, and WSPC with WSU were similar, and the lowest degradation rate was achieved with the WS. The four feed mixtures (WSUPE, WSUPC, WSPE, WSPC), to degradation of crude protein showed the highest proportionate loss after incubation 8 h. The other two feed mixtures (WS, WSU) showed the lowest proportionate loss in crude protein prodegradability. The WSPE and WSUPC treatments had the highest CP ruminal degradation after 16 h ($P < 0.01$). The *in-situ* ruminal degradation of CP was higher ($P < 0.01$) in the WSUPC mixture than other all groups after 24 h, and was similar ($P > 0.05$) one another WSUPE, WSPE and WSPC. On the other hand, observed the lowest degradation value in WS group. Including different fungis and urea the four feed mixtures (WSUPE, WSUPC, WSPE, WSPC), for crude protein showed the highest degradation after incubation 48 h. The WSUPE feed mixture showed peak crude protein degradation in ruminal incubation after 72 h and 96 h (80.32%, 90.82 % respectively), which were higher ($P < 0.01$) than the corresponding findings for the *P. chrysosporium* + urea treatment and other groups.

Table 3. Effect of mushroom inoculation and urea treatment on dry matter and crude protein rumen degradability at the end of the incubation times.

Feed Mixture Groups	Incubation times (hour)															
	0		4		8		16		24		48		72		96	
	DM	CP	DM	CP	DM	CP	DM	CP	DM	CP	DM	CP	DM	CP	DM	CP
	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
WSPE	11.22^{a}	9.61^{a}	18.46^{a}	17.62^{a}	26.23^{a}	24.62^{a}	34.42^{a}	35.60^{a}	41.02^{c}	43.79^{b}	57.71^{b}	65.21^{a}	70.59^{b}	75.41^{c}	76.44^{ab}	84.31^{c}
WSUPE	9.69^{b}	8.63^{b}	15.91^{b}	14.88^{bc}	24.31^{ab}	25.53^{a}	33.62^{a}	33.86^{b}	42.68^{bc}	44.37^{b}	62.35^{a}	66.47^{a}	70.71^{b}	80.32^{a}	79.24^{a}	90.82^{a}
WSPC	7.99^{c}	6.72^{c}	15.32^{b}	13.86^{cd}	26.13^{a}	24.77^{a}	34.30^{a}	32.85^{b}	46.32^{a}	44.19^{b}	63.25^{a}	65.11^{a}	72.61^{a}	75.33^{c}	77.45^{a}	82.86^{c}
WSUPC	8.29^{c}	8.70^{b}	15.83^{b}	15.36^{b}	25.82^{a}	25.54^{a}	33.90^{a}	35.78^{a}	44.54^{ab}	45.96^{a}	62.22^{a}	65.58^{a}	72.43^{a}	78.49^{b}	78.67^{a}	88.11^{b}
WSU	8.62^{bc}	7.54^{c}	14.51^{bc}	13.23^{d}	22.91^{b}	22.33^{b}	30.35^{b}	29.70^{c}	38.34^{d}	38.56^{c}	54.24^{c}	55.67^{b}	67.59^{c}	69.37^{d}	74.10^{bc}	76.09^{d}
WS (c)	7.62^{c}	5.44^{d}	12.99^{c}	9.46^{e}	19.76^{c}	15.46^{c}	26.86^{c}	24.80^{d}	34.01^{e}	32.55^{d}	49.64^{d}	50.16^{c}	62.41^{d}	63.38^{e}	71.80^{c}	71.85^{e}
SEM	±0.39	±0.29	±0.64	±0.44	±0.79	±0.56	±0.58	±0.59	±0.76	±0.50	±0.76	±0.85	±0.57	±0.59	±0.97	±0.71

DM: dry matter; CP: crude protein; WSPC: wheat straw + Phanerochaete chrysosporium; WSUPC: wheat straw + urea + Phanerochaete chrysosporium; WSPE: wheat straw + Pleurotus eryngii; WSUPE: wheat straw + urea + Pleurotus eryngii; WSU: wheat straw + urea; WS: wheat straw; c: control; SEM: standard error of the mean. **: P<0.01; a-e: means in the same column with different letters differ

***In situ* degradability parameters**

Statistically analysis of *in-situ* degradation parameters for DM and CP in the study is presented in Table 4. Differences were found significant ($P < 0.01$) among the different treatments "a" parameter for DM. The “a” parameter was higher in WSPE group than other feed mixture groups and other groups were similar one another statistically. Not significant differences were observed the ‘b’ fraction for DM in the different treatments (Table 4). Rumen passage rate of DM fraction ‘c’ varied among feed mixtures groups, and the ‘c’ value for DM in WSPC was higher ($P < 0.01$) than other groups (Table 4). The lowest passage rate value DM fraction was obtained from WS (control) group. Significant differences were observed among the treatment groups in terms of the ‘k’ fraction. WSUPE, WSPC, WSUPC feed mixture groups were the highest DM fraction ‘k’ parameter value.

Differences were found among the different treatments for *in-situ* ruminal degradation of CP; the differences in the ruminal degradation of those parameters were similar partially with findings of DM in terms of interaction among groups. The *in situ* ruminal degradation of ‘a’ fraction for CP was higher in WSPE group than other feed mixture groups. As to the ‘b’ fraction was the highest in the WSUPE group. Results of the study revealed that in feed mixture groups, uninoculed and inoculed wheat straw have rumen passage rate fraction (c) 0.01 to 0.02 for CP degradability. WSPE, WSPC, WSUPC and WSU groups were similar statistically and had the highest ‘c’ fraction value. There was significant difference among other groups of treatments with these groups ($P < 0.01$). The “k” fraction was the highest in WSUPE group for CP degradability.

Table 4. Effect of mushroom inoculation and urea treatment on dry matter and crude protein degradability parameters at the end of the incubation times.

Feed Mixture Groups	Degradability parameters							
	a (%)		b (%)		c (1/h)		k 0.05/h	
	DM	CP	DM	CP	DM	CP	DM	CP
	**	**	ns	**	**	**	**	**
WSPE	12.66[a]	10.37[a]	75.08	83.59[bc]	0.021[ab]	0.02[a]	63.15[b]	63.68[c]
WSUPE	9.82[b]	9.25[ab]	77.25	97.25[a]	0.024[ab]	0.01[b]	63.31[a]	64.0[a]
WSPC	8.19[b]	7.15[c]	73.51	83.25[bc]	0.030[a]	0.02[a]	63.40[a]	63.62[c]
WSUPC	9.07[b]	9.33[ab]	75.24	88.75[b]	0.026[ab]	0.02[a]	63.37[a]	63.96[b]
WSU	9.60[b]	8.34[b]	77.02	81.69[c]	0.020[ab]	0.02[a]	62.98[c]	63.08[d]
WS (c)	7.80[b]	5.06[d]	82.10	85.55[bc]	0.015[b]	0.01[b]	62.86[c]	62.87[e]
SEM	±3.65	±0.37	±2.31	±2.01	±0.001	±0.001	±0.047	±0.034

a: fast dissolution and degradation in rumen for nutrients. b: potential degradability value that requires a certain time to dissolve and degrade. c: from rumen passage rate that is assumed as 0.02/h. t is degradation time. k is flow rate from rumen. DM: dry matter; CP: crude protein; WSPC: wheat straw + Phanerochaete chrysosporium; WSUPC: wheat straw + urea + Phanerochaete chrysosporium; WSPE: wheat straw + Pleurotus eryngii; WSUPE: wheat straw + urea + Pleurotus eryngii; WSU: wheat straw + urea; WS: wheat straw; c: control; SEM: standard error of the mean. **: P<0.01; ns: no significant; a-e: means in the same column with different letters differ

Discussion

In the present study, the WSPC treatment had a higher value in terms of DM content after 30 d incubation than the other treatment groups. It was determined which was in agreement with the founding of those Karimi et al. (2014) (untreated rice straw), Shrivastava et al., (2011), Denek and Deniz (2004). But it was indicated more high findings by Kutlu et al., (2000) and more low findings (maize stalk) by Tao et al., (2016). The high ratio of DM in WSPC treatment group can probably explained by the fact that some fungis behave according to the acidic, alkaline or neutral environment of the environment and may affect the water retention and weight losses of the material they are inoculated (Talaei et al., 2013). Thus, they react to heat treatment differently. Because of these properties, they are thought to maintain the bound moisture of the material in which they are inoculated and thus prevent weight loss (Talaei et al., 2013). In the present study in chemical analysis, dry matter content of urea added mixtures (WSUPC, WSUPE) was showed lower than the form (WSPC, WSPE) without urea of the same mixture group. This situation was thought to be caused the fungi to increase of nutrient losses in urea added environment. Thus, it was reported that the loss of dry matter in fungal processes results from increase of consumption of carbohydrates by the fungi (Shrivastava et al., 2011; Chaturvedi and Verme 2013; Sharma and Arora 2013).

The WSUPE group had the highest crude protein content among the feed mixture groups. This result determined in the present study had a higher value than from results reported by Jalc et al., (1997), Karimi et al., (2014) and Kutlu et al., (2000) which carred out urea molasses treated rice straw. Fungal inoculation had been reported to increase protein content (Zadrazil and Uniya, 1995). However, it is possible to encounter different results. Crude protein content was higher in fungal and urea added mixtures (WSPC, WSUPC, WSPE, WSU) than control group. This result agreed with finding reported by Jalc et al., (1997).

In this present study, the hemicellulose, ADF, NDF, ADL and cellulose content of the WSU and WS treatment groups had peaks generally (except WSU for NDF, inclusive WSUPE for cellulose). Yao and Nokes (2014) and Singh et al., (2011) investigated wheat straw treated with *P. chrysosporium* and showed a significant drop in this cell wall components comparison to the untreated straw after incubation. The levels of ADF in the all mixture feed groups were also lower in comparison to results reported by Jalc et al., (1997) and Adamovic et al., (1998). On the other hand, the NDF (Jalc et al., 1997) and cellulose (Adamovic et al., 1998) values of the mixture groups obtained by inoculating the fungi to straw found lower from results of different studies. Similar effects on the hemicellulose value of wheat straw were presented by (Adamovic et al., 1998). Fungal treatment significantly reduced the concentration of the cellwall components (NDF, hemicellulose) using the fungi PE and (ADF, cellulose) using the fungi PC.

The results with respect to in initial incubation times (until 16 h) dry matter degradability in rumen showed that without urea treatment. PE fungi inoculation had significant effects on initial dry matter degradability. Urea adding treatment increased partially initial dry matter degradability in this

incubation times. Although PC fungi inoculation did not affect in high ratio dry matter degradability in urea treated straw in initial incubation times, inlater incubation times (after 24 h) increased dry matter degradability. This stuation is mainly a reflection of action of fungi in the alkali condition in urea treated straw. Dry matter degradation in rumen increased with urea treatment. fungi inoculation and the increase in the incubation times. After 24 hours rumen incubation, it was observed that was affect of WSPC and WSUPC groups high degradability in rumen in term of dry matter in 48, 72 and 96 h. Similarly, Kutlu et al., (2000) reported that dry matter degradation rates of treatment groups at 24, 48, 72 and 96 h was affected by the urea treatment, mushroom inoculation, the time after mushroom inoculation and urea x mushroom interaction. Likewise it was stated increased rumen degradability with incubation time (Moon et al., 2010), degraded approximately 25% (Yıldırım and Yıldız, 2010) and 80% (Valmaseda et al., 1990) of DM by PE fungi. On the other hand, there were no significant differences between untreated and treated rice straw in terms of DM and NDF degradability was reported by Karimi et al., (2014). Also, it was indicated straw treated with PE fungus showed the lowest degradability at 48 th, 72 th and at 96 th h of incubation (Fazaeli et al., 2004).

In the study, rapidly soluble DM fraction (a) varied among feed mixture groups. and the ‘a’ value for DM in WSPE group was higher both numerical and statistical than other forages (Table 3). The lowest rapidly degradable DM fraction was obtained, not statistical but numerical, from WS (control) group. The average rapidly soluble DM fraction for all feed mixture groups in this study was lower than the findings reported by Turgut and Yanar (2004) for some forages and by Karimi et al., (2014) for treated rice straw. But, the average ‘a’ value for all feed mixture groups in this study was higher generally than findings of Tao et al., (2016) and Karimi et al., (2014) determined for untreated rice straw. These discrepancies may be attributed to content of the feed mixture groups and substrat differences in the studies.

Although the potential degradability fraction ‘b’ values for DM were not significant among groups statistically, it was observed difference at a certain rate among mixture groups (Table 4). The degradable DM fraction value of ‘b’ for different substrat reported by Tao et al., (2016), Karimi et al., (2014) and Turgut and Yanar (2004) were lower than the ‘b’ fraction value obtained in the present study.

The rumen passage rate of DM degradation (c value) in the feed mixture groups were high in groups treated with urea and fungi, Karimi et al., (2014) was determined similar reseults in untreated rice straw and urea molasses treated rice straw groups. The higher results were obtained by Turgut and Yanar (2004) who reported ‘c’ values for different forages. Tao et al., (2016) found partly lower values for the ‘c’ fraction in cell-wall components in the maize stalk according to this research findings.

Significant differences were observed among feed mixture groups studied with respect to effective degradabilities of DM. The result was in accordance statistically with finding of Tao et al., (2016) and Turgut and Yanar (2004) but were the higher numerically from results of these two studies.

In the study, the crude protein content was significantly increased by fungal inoculation and urea treatment and this increase had changed accorgin to fungi varieties. Urea treatment and incubation times. In initial incubation times (until 8 h) crude protein degradability in rumen showed that without urea treatment. PE fungi inoculation had obvious effects on initial crude protein degradability. It was no significant difference among the groups until the 8 th hour but after from 16 th hour this interaction began to become clear. At the 16 th hour, the WSUPC group showed high protein degradability associated with WSPE group. Similar situation was observed at 24 hours as well. Crude protein degradability found higher in groups that treated fungi and urea both statistically and numerically at 48 th hour. Similarly, at 72 th and 96 th hours, the fungus and urea treated groups had high protein degradability and PE fungus was found to be more effective, especially in the urea-treated environment. The assumption increase of protein content by fungal inoculation is one of hypothesis of this study. This hypothesis is supported by Tuyen et al., (2012) and Zadrazil and Uniya (1995). Similar to our results, Turgut and Yanar (2004) found that incubation times increased the CP gain in different forages.

Effect of the feed mixture groups on the degradation rate all the parameters of the CP (a, b, c, k) was appearance similar with DM degradation. Although the mixture groups inoculed with PE had the higher ‘a’ value in initial of incubation in this study, inoculed groups with PC had showed similar effect in the forthcoming days. Means of ‘a’ for all feed mixture groups were lower than findings of Turgut and Yanar (Turgut and Yanar, 2004) who determined degrdation parameters for CP in some forages. However. the "b" parameter value determined for CP in this study were found to be lower than our results. Besides, Tao et al., (2016) showed that the PC types of white rot fungi increased the ‘a’ and ‘b’ parameters value of maize stalk in comparison to without fungi inoculants. In the present study, the results determined for the "a" parameter were higher than the findings indicated by Tao et al., (2016) and the lower ‘b’ parameter. The differences in both ‘a’ and ‘b’ CP values may be as a result of differences in fungus variete, urea treatment, incubation times on wheat straw. The parameter ‘c’ followed a close course among the feed mixture groups. The parameter ‘k’ followed a diferrent course among the feed mixture groups. Similarly, Tao et al., (2016) investigated maize stalk treated with *P. chrysosporium* and showed a significant different (decrease) in the content of without fungus in comparisonto the untreated straw after incubation.

Conclusions

Results of this study suggest that fungal inoculation and urea treatment are promising means to convert low-quality wheat straw into a higher quality ruminant feed. The inoculantion of PC, PE and urea treatment the greater effect on improving the ruminal degradability of DM and CP. The degradation of the DM in wheat straw was altered significantly, mainly by the fungi PC. This fungus can be used in wheat straw for husbandry.

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Conflict of interest

The authors acknowledge that this study is a Ph.D thesis. The authors declared that there is no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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