

Effects of *Pleurotus florida* Inoculation or Urea Treatment on Feeding Value of Wheat Straw*

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Abstract: The present study was conducted to determine whether the mushroom *Pleurotus florida* has potential to increase the digestibility and feeding value of wheat straw in with urea treatment. In the trial the effects of inoculation of *P. florida*, and 2% urea treatment were examined in a 2 by 2 factorial design. The mushroom was inoculated to urea supplemented or unsupplemented wheat straw by solid-state fermentation. Following inoculation, on the 20th, 40th, 60th and 80th days straw samples were taken and analysed for nutrient content and tested for digestibility on sheep using the nylon bag technique.

The results showed that *Pleurotus florida* inoculation increased digestibility and nutrient content of wheat straw ($P<0.05$). *Pleurotus florida* increased digestibility, crude protein, crude oil, nitrogen-free extract about 22, 60, 20, 5%, respectively, while reducing crude fibre content about 16%. Urea supplementation inhibited mushroom growth on the straw. However, urea supplementation to untreated wheat straw increased digestibility and feeding value of the straw significantly ($P<0.05$). In contrast to untreated wheat straw, in the wheat straw supplemented with 2% urea, digestibility, crude protein, crude oil, nitrogen-free extract were increased about 19, 30, 60, 9%, respectively and crude fibre contents were reduced about 20%.

Key Words: Wheat straw, Feeding value, *Pleurotus florida*, urea treatment.

Buğday Samanının Yem Değeri Üzerine *Pleurotus florida* İnokülasyonu ve Üre Muamelesinin Etkileri

Özet: Mevcut araştırma, üre muamelesi ile mukayeseli olarak *Pleurotus florida* türü mantar inokülasyonunun buğday samanının yem değerini üzerine etkisinin belirlenmesi amacıyla yürütülmüştür. Çalışmada, saman üzerine *Pleurotus florida* mantar inokülasyonu ve %2 üre muamelesi 2x2 faktöryel deneme tertibinde test edilmiştir. Mantar, üre muamelesi yapılmış ve yapılmamış buğday samanı üzerinde solid-state fermentasyonla yetiştirilmiş ve mantar ekimini takip eden 20., 40., 60. ve 80. günlerde saman örnekleri, besin madde analizlerine ve canlı hayvan üzerinde naylon-kese yöntemi uygulanarak sindirim denemelerine tabi tutulmuştur.

Çalışmada elde edilen bulgular, üre muamelesiz *P.florida* ekimi ile samanın yem değerinin önemli ($P<0.05$) düzeyde artırılabilceği saptanmıştır. *P.florida* ekimi ile samanın sindirilebilirliği, ham protein, ham yağ ve azotsuz öz maddede içeriğindeki artışın sırasıyla %22, 60, 20 ve 5 olduğu, ham selüloz içeriğindeki azalmanın ise %16 düzeyinde olduğu saptanmıştır. Öte yandan üre muamelesi saman üzerinde mantar gelişimini engellemiş, ancak samanın yem değerini önemli düzeyde artırmıştır. %2 üre muamelesi ile samanın sindirilebilirliği, ham protein, ham yağ ve azotsuz öz madde içerikleri sırasıyla %19, 30, 60 ve 9 oranında artarken, ham selüloz içeriği %20 düzeyinde azalmıştır.

Anahtar Sözcükler: Buğday samanı, yem değeri, *Pleurotus florida*, üre muamelesi.

Introduction

Lignocellulose, whose major components is cellulose, is the most abundant organic material on earth. Straw is the most common lignocellulolytic residue at many farms where cereals are the main crops (1). The importance and usability of straw are variable country to country. In some countries straw obtained after harvesting is burnt in the

field because of the high cost of transportation for utilisation in industries (2,3) or feeding, whereas in other countries straw is a very important raw material for the cellulose industry or as a compound in the preparation of mushroom compost (4,5) or as roughage for ruminants' feeding. Especially in developing countries, where straw is common and the most important roughage source for ruminant animals because of insufficient green feed

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production, improving the feeding value of straw is an important task for ruminant nutrition.

In order to improve the feeding value of straw some chemical methods such as NaOH or urea treatment have been employed with varying degrees of success (6-8). An alternate method for improving the feeding value of straw could be the application of biotechnology. Literature on the degradation of agricultural residue has revealed that inoculation of *Pleurotus* spp., which are known to have lignocellulolytic enzyme activity, could improve the feeding value of straw by degradation of lignin (9, 10).

The present study was conducted to determine whether the mushroom *Pleurotus florida* has potential to increase the digestibility and feeding value of wheat straw in comparison with urea treatment.

Materials and Methods

Wheat straw used in the experiment was obtained from Çukurova University, Agricultural Faculty, Research and Application Farm in four bags, each containing 10 kg. Two bags of the straw were soaked and treated with feed grade urea in a ratio of 2% and then dried, while the other bags of the straw were kept untreated.

The mushrooms, *Pleurotus florida* micelium, which were inoculated with straw, were obtained from Yalova Horticultural Institute of Turkey. The micelium were grown at the Dept. of Horticultural Sci. of the Agricultural Faculty, Çukurova University, according to the method of Abak (11) and then inoculated with half of urea treated or half of untreated wheat straw in the ratio of 65g per kg straw, while the other half were used as urea treated or untreated controls. As a result of these treatments (2 by 2 factorial design), 4 groups of straw were prepared, these were 1: untreated straw, 2: mushroom (*Pleurotus florida*) inoculated straw, 3: urea treated straw and 4: urea treated-mushroom (*Pleurotus florida*) inoculated straw.

Mushroom inoculated urea treated or untreated wheat straw samples were kept in a dark room at 23-25°C and 80% relative humidity. Following the inoculation, on day 20, 40, 60 and 80, straw samples from all groups were taken and analysed for nutrient content (dry matter, crude protein, crude ash, crude fat, fibre and nitrogen free extract) according to the methods of AOAC (12). The straw samples were also analysed for dry matter digestibility in the rumen using the nylon bag (in situ) technique in sheep according to the method of Orskov (13). Degrees of dry matter degradability were

measured after incubation in the rumen with 24 hour intervals for 72 hours. Initial dry matter loss was measured by washing the sample with tap water for 45 minutes.

For the digestibility studies, two 50 kg live weight, 12-month-old fistulated male sheep were used. The nylon bags used throughout the digestibility studies were obtained from the Rowett Research Centre in the UK. Each nylon bag was produced with 8 cm width, 11 cm length using 12 micron square pore sized special nylon cloths. During the digestibility studies the animals were fed with good quality alfalfa hay and water *ad libitum*.

The data obtained in the experiment were analysed using ANOVA (two-way variance analyses) procedure of SAS (14) and means were separated using Duncan's New Multiple Range Test.

Results and Discussion

Nutrient Content

In order to determine the effects of mushroom inoculation and/or urea treatment on the nutrient content, following the inoculation, on day 20, 40, 60 and 80, straw samples from all groups were analysed for dry matter, crude protein, crude ash, crude fat, fibre and nitrogen free extract.

The results with respect to dry matter content showed that urea treatment, mushroom inoculation and the time (day) after mushroom inoculation and also their interactions had no significant ($P>0.05$) effect on the dry matter content of straw and all the groups exhibited similar dry matter values (Table 1).

Similarly, there were no significant ($P>0.05$) effects of urea treatment, mushroom inoculation or the time (day) after mushroom inoculation and also their interactions on crude ash content of straw and straw from all the groups exhibited similar crude ash values (Table 1).

However, the crude protein value of straw was markedly affected by urea treatment, as expected, and by mushroom inoculation, the time (day) after mushroom inoculation and also Urea x mushroom interaction ($P<0.01$). The crude protein content of straw increased as time passed after the mushroom inoculation and the highest value was obtained on day 80 (Table 1). However, in the urea treated straw samples there was no effect of mushroom inoculation noted. This was mainly due to the lack of growth of mushroom in the alkali condition. In fact no growth of mushroom was observed in urea

Table 1. Effect of mushroom inoculation and urea treatment on dry matter, crude ash and crude protein contents of straw.

Time after <i>P. florida</i> inoculation(day)	Treatments				Mean
	Straw	Straw + <i>P. florida</i>	Straw + Urea	Straw + Urea + <i>P. florida</i>	
Dry Matter (%)					
20	95.73 ± 0.20	96.62 ± 1.40	97.20 ± 0.49	96.73 ± 0.53	96.57a
40	95.71 ± 0.19	95.09 ± 0.01	95.53 ± 0.36	94.68 ± 0.59	95.25a
60	95.71 ± 0.19	95.03 ± 0.02	94.81 ± 0.01	94.55 ± 0.02	95.03a
80	95.71 ± 0.19	93.60 ± 0.20	94.56 ± 0.22	93.34 ± 0.02	94.30a
Mean	95.72 a	95.09 a	95.52 a	94.83 a	
Urea (no/yes)		95.40a		95.17a	
<i>P. florida</i> (no/yes)		95.62a		94.96a	
Crude Ash(%)					
20	8.19 ± 0.40	8.09± 0.11	8.34±0.52	8.54 ± 0.08	8.29a
40	7.79 ± 0.01	8.45±0.12	7.91±0.23	8.09 ± 0.46	8.07a
60	7.84 ± 0.14	8.25±0.41	7.88± 0.01	7.95 ± 0.27	7.98a
80	7.84 ± 0.14	8.21±0.25	8.39 ± 0.15	8.13 ± 0.23	8.14a
Mean	7.91a	8.26a	8.13a	8.18a	
Urea (no/yes)		8.09a		8.15a	
<i>P. florida</i> (no/yes)		8.02a		8.22a	
Crude Protein (%)					
20	3.97 ± 0.01	5.64± 0.03	6.08 ± 0.17	6.10 ± 0.09	5.45c
40	3.95 ± 0.01	6.46± 0.14	6.46 ± 0.25	6.44 ± 0.13	5.83b
60	3.95 ± 0.02	6.61± 0.23	6.70 ± 0.25	6.73 ± 0.04	5.99ab
80	3.95 ± 0.04	6.85± 0.10	6.73 ± 0.20	6.71 ± 0.09	6.06a
Mean	3.96 b	6.39a	6.49a	6.50a	
Urea (no/yes)		5.17b		6.49a	
<i>P. florida</i> (no/yes)		5.23b		6.44a	

* : for the same parameter, on the same row or on the same column means with different letters are significantly different ($P < 0.05$).

treated straw samples. Günay (15) reported that the optimum pH for the growth of *Pleurotus* spp. is 6.4-7.0, however 2% urea treatment in this experiment increased the pH to 8.8, leading to intolerable conditions for normal growth of the mushroom.

The results with respect to crude oil content revealed that mushroom inoculation, urea treatment and the time after mushroom inoculation had no significant effect. However, a significant effect of urea x mushroom interaction was observed, whereby the crude oil content of untreated straw was increased ($P < 0.05$) by mushroom inoculation, although similar effects were not detected in urea treated straw. This could also be attributed to the lack of growth of mushroom in the alkali condition.

The results obtained in the experiment showed that the crude fibre content of straw samples was markedly affected ($P < 0.01$) by urea treatment, mushroom inoculation, the time (day) after mushroom inoculation and urea x mushroom interaction. Mushroom inoculation and urea treatment reduced the crude fibre content of straw considerably. The positive effect of mushroom inoculation increased as time passed after the inoculation and the lowest value was obtained on day 80 (Table 2). Urea treatment with or without mushroom inoculation also induced a marked reduction in the crude fibre content of straw. The above mentioned positive effects can be attributed to the lignocellulolytic enzyme activity of the mushroom *Pleurotus florida* (9, 10) and also the alkali activity of urea (16, 17). Although both activities

work in different mechanisms, the first by enzyme, the second by chemical hydrolyzation, the net results are similar. However, both activities could not be combined as the mushroom cannot grow in alkali conditions.

It should be noted that the nitrogen free extract content of straw was calculated by subtracting crude protein, crude ash, crude oil and crude fibre from dry matter content. As a result of changes in the nutrient content of straw by mushroom inoculation and/or urea treatment, the nitrogen free extract content of straw was also changed. The changes in the nitrogen free extract content were mainly induced by urea treatment (Table 2). A significant effect of urea x mushroom interaction was also noted. Urea treatment on mushroom inoculated or plain straw increased the nitrogen free extract content

markedly ($P < 0.01$). However, mushroom inoculation enhanced the nitrogen free extract content in only untreated straw not in urea treated straw. This interaction can again be attributed to the lack growth of mushroom, as mushroom did not grow on 2% urea treated straw.

Rumen Degradation

The results with respect to initial dry matter loss showed that urea treatment, mushroom inoculation, the time after mushroom inoculation and their interactions had significant effects on initial dry matter loss (Table 3). Initial dry matter loss increased ($P < 0.05$) with the time after mushroom inoculation until day 60. Urea treatment and mushroom inoculation also increased initial dry matter loss. Although mushroom inoculation did not

Table 2. Effect of mushroom inoculation and urea treatment on crude oil, crude fibre and nitrogen free extract contents of straw.

Time after <i>P. florida</i> inoculation(day)	Treatments				Mean
	Straw	Straw + <i>P. florida</i>	Straw + Urea	Straw + Urea + <i>P. florida</i>	
Crude oil (%)					
20	2.05 ± 0.10	2.39 ± 0.20	2.27 ± 0.19	2.12 ± 0.13	2.21 a
40	2.04 ± 0.16	2.46 ± 0.14	2.16 ± 0.05	2.17 ± 0.19	2.21 a
60	2.13 ± 0.21	2.51 ± 0.17	2.15 ± 0.20	2.18 ± 0.09	2.24 a
80	2.10 ± 0.19	2.50 ± 0.25	2.13 ± 0.20	2.06 ± 0.25	2.20 a
Mean	2.08 b	2.46 a	2.18 b	2.13 b	
Urea (no/yes)		2.27a		2.15a	
<i>P. florida</i> (no/yes)		2.13a		2.30a	
Crude Fibre (%)					
20	36.63 ± 0.15	33.56 ± 0.44	31.20 ± 0.10	31.18 ± 0.09	33.14 a
40	37.13 ± 0.65	31.12 ± 0.11	29.53 ± 0.34	29.25 ± 0.20	31.76 b
60	37.62 ± 0.02	30.74 ± 0.76	29.32 ± 0.29	29.81 ± 0.09	31.87 b
80	37.71 ± 0.32	30.62 ± 1.23	29.38 ± 0.23	29.32 ± 0.46	31.76 b
Mean	37.27 a	31.51 b	29.89 c	29.86 c	
Urea (no/yes)		34.39a		29.88 b	
<i>P. florida</i> (no/yes)		33.57a		30.70 b	
Nitrogen Free Extract (%)					
20	44.89 ± 0.66	46.94 ± 0.90	49.31 ± 1.26	48.81 ± 0.52	47.49 a
40	44.81 ± 0.66	46.56 ± 0.30	49.46 ± 0.01	48.72 ± 0.92	47.39 a
60	44.17 ± 0.16	46.92 ± 0.30	48.78 ± 0.22	47.88 ± 0.57	46.93 a
80	44.11 ± 0.50	45.43 ± 1.45	47.82 ± 0.37	47.10 ± 0.88	46.14 a
Mean	44.50 c	46.46 b	48.62 a	48.13 a	
Urea (no/yes)		45.48 b		48.49 a	
<i>P. florida</i> (no/yes)		46.68 a		47.29 a	

* : for the same parameter, on the same row or on the same column means with different letters are significantly different ($P < 0.05$).

Table 3. Effect of mushroom inoculation and urea treatment on initial dry matter lose and rumen degradability at the end of the 24, 48 and 72 hours incubation.

Time after P. florida inoculation(day)	Treatments				Mean
	Straw	Straw + P. florida	Straw + Urea	Straw + Urea + P. florida	
Initial dry matter lose					
20	11.88±0.31	15.32±0.11	16.53±0.07	16.60±0.25	15.08c
40	11.79±0.23	17.87±0.32	18.35±0.12	19.74±0.19	16.94b
60	11.82±0.21	17.43±0.13	19.56±0.21	20.27±0.15	17.27a
80	11.93±0.18	17.25±0.18	20.05±0.28	19.97±0.20	17.30a
Mean	11.85 c*	16.97 b	18.62 a	19.15 a	
Urea (no/yes)		14.41b		18.86 a	
P. florida (no/yes)		15.24 b		18.06 a	
Dry matter Degradability after 24 h. incubation in the rumen					
20	40.24±1.40	46.37±0.99	51.60±0.33	51.03±0.40	47.31 b
40	42.16±0.53	50.59±1.53	55.74±1.76	55.44±2.06	50.98 a
60	42.60±0.53	46.78±0.83	54.73±1.11	53.84±1.33	49.49 a
80	42.21±0.42	46.78±1.08	55.44±1.22	56.32±1.17	51.04 a
Mean	42.30 c	47.99 b	54.37a	54.16 a	
Urea (no/yes)		45.14b		54.27 a	
P. florida (no/yes)		48.34 b		51.07a	
Dry matter Degradability after 48 h. incubation in the rumen					
20	49.25±1.50	56.48±0.93	60.77±0.34	60.83±0.67	56.83 a
40	47.74±0.54	56.41±0.90	64.68±1.30	64.15±0.84	58.24 a
60	49.40±1.02	52.76±0.47	63.65±0.78	63.63±0.77	57.36 a
80	49.23±0.43	54.14±0.48	62.21±0.43	62.42±0.55	57.00 a
Mean	48.90 c	54.95 b	62.83 a	62.76 a	
Urea (no/yes)		51.93b		62.79a	
P. florida (no/yes)		55.87b		58.85a	
Dry matter Degradability after 72 h. incubation in the rumen					
20	53.58±0.44	64.84±0.80	67.49±0.13	65.55±0.28	63.32ab
40	53.04±0.29	65.66±0.68	68.81±0.44	68.84±0.48	63.88a
60	52.65±0.70	58.39±0.25	69.44±0.49	69.28±0.12	62.44 c
80	53.36±0.18	58.87±1.23	69.11±0.26	69.45±0.17	62.70bc
Mean	53.16c	61.94b	68.53a	68.71a	
Urea (no/yes)		57.55b		68.62a	
P. florida (no/yes)		60.94b		65.23a	

* : for the same parameter, on the same row or on the same column means with different letters are significantly different (P<0.05).

affect initial dry matter loss in urea treated straw, mushroom inoculation increased (P<0.05) initial dry matter loss in untreated straw. This interaction is mainly a reflection of the lack of growth of mushroom in the alkali condition in urea treated straw.

The results obtained with respect to rumen degradability showed that incubation time in the rumen had a significant effect, therefore, rumen degradability in each incubation time was given and discussed separately.

At the end of 24 hours rumen incubation, it was observed that rumen degradability was affected ($P<0.01$) by the urea treatment, mushroom inoculation, the time after mushroom inoculation and urea x mushroom interaction. Rumen degradability increased with urea treatment, mushroom inoculation and the increase in the time (day) after mushroom inoculation. It was also observed that the effect of mushroom on 24 hour degradability was much more evident in untreated straw than urea treated straw.

Rumen degradability at the end of 48 hours was affected ($P<0.01$) by the urea treatment, mushroom inoculation, the time after mushroom inoculation x urea and urea x mushroom interactions. Urea treatment and mushroom inoculation markedly increased dry matter degradability, but the positive effect of mushroom was more pronounced in untreated straw. These results also showed that the effect of mushroom on rumen degradability of dry matter was obscured by urea treatment because of inducing high pH.

With respect to 72 hours incubation in the rumen, dry matter degradability was affected by urea treatment, mushroom inoculation, the time (day) after inoculation, day x urea, day x mushroom, urea x mushroom and day x urea x mushroom interactions significantly ($P<0.01$). Rumen degradability was found to be higher on day 20 and 40 than on day 60 and 80. Either urea treatment or mushroom inoculation increased the degradability. However, the positive effect of mushroom inoculation was much more apparent on day 20 and 40. Similar to the 24 and 48 hours rumen incubation, at the end of 72 hours incubation mushroom inoculation improved the degradability in untreated straw, while it had no significant effect in urea treated straw.

In summary, *Pleurotus florida* inoculation in this experiment increased the nutrient content and digestibility of wheat straw. These results are in agreement with the early findings (9, 10, 18). In contrast

to plain straw, mushroom inoculation increased digestibility, crude protein, crude oil, nitrogen-free extract by about 22, 60, 20, 5%, respectively, while crude fibre contents were reduced by about 16%. Urea supplementation inhibited mushroom growth on the straw.

Günay (15) reported that a high pH level, over 7, is not suitable for the growth of *Pleurotus* spp. This may suggest that *Pleurotus* spp. and urea treatment could not be combined in terms of the improving feeding value of straw. However, Gupta (19) reported that some mushrooms like *Coprinus* spp. grow at high pH level and bind excess ammonia released from urea. Inoculation of ammonia binding mushrooms could be, therefore, combined with urea treatment to improve the feeding value of straw. It is also evident that urea supplementation to straw increased the digestibility and feeding value of the straw. In contrast to untreated wheat straw, in the wheat straw supplemented with 2% urea digestibility, crude protein, crude oil, nitrogen-free extract were increased about 30, 60, 9%, respectively and crude fibre contents were reduced about 20%. These results are in agreement with the reports of Arnason and Mo (6), Homb et al. (7), Orskov, (16, 17) and Tuncer et al. (8).

The results obtained in this experiment suggest that mushroom (*Pleurotus florida*) has the potential to improve the digestibility and feeding value of wheat straw. Although urea treatment has similar potential, mushroom treatment could be less harmful to the environment, as the urea treatment could contribute to environmental pollution.

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