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Research Article

Effect of Using Fungal Treated Rice Straw in Sheep Diet on Nutrients Digestibility and Microbial Protein Synthesis

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Abstract

Background and Objective: Biological methods by using white-rot fungi is one of the viable alternatives to improve the nutritional value of rice straw. Moreover, biological method is environmental friendly and potentially economic. The objective of this study was to evaluate the effect of utilisation of fungal treated rice straw in the diet of sheep on feed intake and nutrient digestibility when compared to untreated or urea treated rice straw. **Materials and Methods:** A total of 12 male Phan Rang sheep with body weight (20.58±1.16 kg) were randomly assigned to either a Rice Straw (RS) or a urea treated rice straw (UTR) or fugal treated rice straw (FTR) diet over an experimental period of 20 days in a completely randomized design. The RS diet was a mixture of rice straw, guinea grass and concentrates. In the UTR and the FTR diets, rice straw was replaced by urea treated rice straw and fungal treated rice straw, respectively. During 14 days of adaptation, all sheep were kept in individual pens and received their assigned diets *ad libitum*. During the following 6 days, the sheep were offered at 95% of *ad libitum* intake and moved to metabolism crates for total urine and fecal collection to evaluate feed intake, nutrients digestibility and N balance. All data were tested by ANOVA using the general model procedure of SAS. **Results:** The nutrient intake did not differ among three diets. However, apparent nutrient digestibility were highest ($p<0.05$) in sheep fed the FTR diet. The nitrogen retention and microbial protein synthesis were also greatest (4.32 and 6.04 g/day, respectively, $p<0.05$) for sheep fed the FTR diet. **Conclusion:** These results suggest that using fungal treated rice straw in the diet of sheep showed the best improvement of nutrient digestibility, N retention and microbial protein synthesis.

Key words: *Pleurotus eryngii*, fungal treated rice straw, nutrient digestibility, N retention, sheep

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ruminant livestock plays a crucial role and is an integral part of farming and rural life of tropical countries. It provides food, family income and employment for farmer in rural area[1]. Rice straw is one of the agricultural by-products that is abundantly available in many tropical countries. Small holder farmers usually store it and use rice straw as ruminant feed throughout the year especially in the dry winter season. Although, ruminants are able to convert low quality agriculture by-products into high quality protein but the use of rice straw as ruminant feed is hampered by its low nutritional value such as high Neutral Detergent Fiber (NDF) and lignin content, low protein content and their low digestibility[2].

In 2017, Vietnam produced approximately 44.1 million t of rice. If it is assumed that the ratio of grain to straw is 1:0.8 with 50% collectable rice straw, a total of about 17.64 million t of rice straw would be available as a feed source for ruminants. A daily intake of 5 kg rice straw per head is assumed, the total amount of rice straw would be sufficient to feed more than 9.7 million heads of buffaloes and cattle for a year. Therefore, improving the nutritional value of rice straw is very beneficial in the development of ruminant production in Vietnam, especially in the dry winter season when the amount of forage feed for ruminants is scarce.

Different technologies have been developed to improve the nutritional value of rice straw. Physical and chemical treatments have been reported to increase the intake and digestibility of rice straw and hence the performance of cattle[3]. However, physical and chemical treatments can be expensive, harmful to users or unfriendly to the environment[4]. Biological methods by using white-rot fungi is one of viable alternatives to improve the nutritional value of rice straw. This method is environmentally friendly and potentially economic[4]. Akinfemi and Ogunwole[5] used *Pleurotus ostreatus*, *Pleurotus pulmonarius* and *Pleurotus tuber-regium* to incubate with rice straw for 21 days. The results showed that Crude Protein (CP) content was increased from 4.67-7.69% Dry Matter (DM). In addition, Crude Fiber (CF) and Acid Detergent Lignin (ADL) were reduced from 32.89-19.96% DM and from 12.54-9.68% DM, respectively in all fungal treated rice straw when compared to untreated rice straw. The improvement of nutritional value of rice straw after incubation with these fungi increased the Organic Matter (OM) digestibility from 51.17-57.02% *in vitro* trial[5]. A reduced ADL content and improved CP content of fungal treated rice straw was also found in the study of Jafari *et al*.[6]. In their study, the authors also incubated rice straw with *Pleurotus* fungi for 18 days. Jafari *et al*.[6] reported that *in vitro* DM and OM digestibility were increased by 12.99% (67.11-80.10%) and 26.02% (56.16-82.18%), respectively when compared to untreated rice straw. After testing several strains of *Pleurotus* it found that the nutritional value of rice straw in Vietnam was most effectively increased by reducing ADL content and improving CP contents of rice straw when incubated with *Pleurotus eryngii* for 4 weeks. Moreover, the use of fungal treated rice in the diet of ruminants is still very limited. Therefore, the objective of this study was to further evaluate the effect of utilisation fungal treated rice straw in the diet of sheep on feed intake and nutrients digestibility.

MATERIALS AND METHODS

Animal care: The experimental procedure was approved by the Animal Care and Use Committee at Goat and Rabbit Centre Research, Son Tay, Vietnam.

Dietary treatments: Urea treated rice straw (UTR) was prepared according to methods of Wanapat *et al*.[7] Fungal treated rice straw (FTR) was prepared according to Tuyen *et al*.[4] and Fazaeli *et al*.[8]. *Pleurotus eryngii* (strain MES 03757) from the Laboratory of Wageningen UR Plant Breeding, the Netherlands, was used to inoculate the rice straw in the current experiment.

Guinea grass (*Panicum maximum*) was grown at the experimental facilities of the Goat and Rabbit Centre Research, Son Tay, Vietnam. Guinea grass was fresh harvested daily at 40-45 days after regrowth during experiment period. The chemical compositions of guinea grass, rice straw, urea treated rice straw, fungal treated rice straw and concentrates were included in Table 1.

The rice straw diet was composed of rice straw (333.3 g kg^{-1} of DM), guinea grass (333.3 g kg^{-1} of DM), concentrates (333.3 g kg^{-1} of DM) prepared as a Total Mixed Ration (TMR) diet. In the urea treated rice straw (UTR) diet, total rice straw was replaced by the urea treated rice straw. In the fungal treated rice straw (FTR) diet, total rice straw was replaced by the fungal treated rice straw (Table 1). The TMR were prepared daily. During the diets preparation, samples of rice straw, UTR, FTR, guinea grass and concentrates were taken then pooled after finishing the experiment and stored at -20°C. Diet formulation per treatment was identical for the whole experimental period. The chemical composition of the dietary treatments is given in Table 1. The dietary treatments were formulated by standard of the Goat and Rabbit Centre Research, Son Tay, Vietnam to meet nutrient requirements of the sheep.

Experimental design, animals and management: The experiment was conducted from January-February, 2018 at the Goat and Rabbit Centre Research, Son Tay, Vietnam. The experiment followed a completely randomized design with 3 dietary treatments. A total of 12 male Phan Rang sheep (4 sheep per treatment) with body weight of 20.58 kg (±1.16 kg) were used in this experiment. Before the start of feeding experiments, the animals were dewormed following the standard of the Goat and Rabbit Centre Research, Son Tay, Vietnam. All sheep were first allowed to adapt to the rice straw diet (Table 1) for 10 days prior to the start of the experiment. Subsequently, sheep were randomly assigned to receive either a Rice Straw (RS), urea treated rice straw (UTR) or fungal treated rice straw (FTR) diet (Table 1) over the experimental periods of 20 days (14 days for adaptation period and 6 days for measurement period). Sheep were housed in individual pens for the adaptation period then transferred to individual metabolism crates for measurement period. Each metabolism crate had a device for collecting feces and urine separately. The sheep received their feed twice daily in equal portions at 06:00 and 16:00 h. During the adaptation of 14 days, all sheep were fed their assigned diets and allowed *ad libitum* intake. During the following 6 days, diets were offered at 95% of *ad libitum* intake per sheep to minimize feed residues. The sheep had access to fresh and clean water and mineral blocks for 24 h per day.

Measurements and sampling: During the last 6 days of measurement period, data of feed offered, feed residues, feces and urine were recorded daily for each sheep. Feed offered as Guinea grass, rice straw, urea treated rice straw, fungal treated rice straw and concentrates were sampled and stored at -20°C before being oven dried at 60°C for 72 h and ground in an cross beater mill to pass through a 1 mm sieve. After grinding, all samples were stored at 4°C pending analysis. Feed ingredient samples were analyzed for DM, ash, nitrogen (N), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL).

Feed residues were collected and weighed twice daily, before the morning and afternoon feeding and stored at 4°C. Residues were pooled per sheep during the last 6 days of measurement period and then subsampled. Feed residue subsamples were oven dried at 60°C and ground in a cross beater mill to pass through a 1 mm sieve before DM analysis. Feed DM intake was calculated by subtracting the dry weight of feed residues from the dry weight of feed offered. Body weight of each sheep was weighed and recorded at the beginning and at the end of the experiment.

The feces excreted by each sheep during 24 h were collected, weighed and stored at 4°C. Total feces were pooled per sheep during the last 6 days of measurement period and 3 subsamples of approximately 500 g for each sheep were collected in sealable containers and immediately stored in a freezer at -20°C. Two of the fecal sub-samples were then oven dried at 60°C, ground at 1 mm sieve and stored at 4°C before analysis of DM, ash, NDF, ADF. The 3rd fecal subsample was thawed overnight to ambient temperature and analyzed for DM and N content in the wet material to determine CP digestibility and N retention. Apparent digestibility of nutrients was calculated by the difference between intake and fecal output of that nutrient.

Urine excreted by each sheep during 24 h was collected in bottle containing 150 mL of 2.5 N H_2SO_4 to reduce pH below 3.0 and prevent bacterial destruction in urine samples and NH_3 losses. Urine volume was weighed (corrected for acid H_2SO_4 adding) and representative sample equivalent to 20% of the total volume was subsampled and immediately stored at -20°C. Urine sub-samples were analyzed for total N, allantoin, xanthine-hypoxanthine and uric acid excretions. Nitrogen retention (g/day) was estimated from N consumed through feed (corrected for feed residues) and excreted in the feces, urine.

Analytical procedures: Content of DM was determined gravimetrically after 4 h drying in a forced air stove[9] at 103°C with ash determined after incineration for 3 h in an oven[10] at 550°C. Nitrogen was determined using the Kjeldahl method with copper (II) sulfate as catalyst[11]. Neutral Detergent Fiber (NDF) was analyzed according to Van Soest *et al*.[12]. Acid detergent fiber (ADF) and ADL were determined according to Van Soest[13]. Allantoin was determined by the method of Young and Conway[14] while xanthine, hypoxanthine and uric acid were analyzed following the method of Fujihara *et al*.[15]. Total urinary PD was calculated as the sum of allantoin, xanthine-hypoxanthine and uric acid excretions. Microbial nitrogen was calculated from total urinary PD excretion based on the quantitative relationship according to Chen and Gomes[16].

Statistical analysis: Effects of dietary treatments on feed intake, nutrients digestibility and N balance were tested by ANOVA using the General Linear Model procedure of SAS[17] following the model:

$$Y = \mu+T_i+\varepsilon_{ij}$$

where, Y is the dependent variable, μ is the overall mean, T_i is the effect of dietary treatments (i = 1-3) and ε_{ij} is the residual error term. The results are presented as the least square means and standard error of the means (LSM±SEM). Differences among main effects were analyzed using Tukey-Kramer's multiple comparison procedure in the LSMEANS statement of SAS[17] with effects considered significant at $p\leq 0.05$ and a trend at $0.05<p\leq 0.10$.

RESULTS

Chemical composition of diets, nutrient intake and digestibility: Since the diet treatments only differ in the type of rice straw, therefore the chemical composition of the diet treatments depends on the chemical composition of the type of rice straw. The CP and ADL contents of FTR were 83.8 g kg^{-1} DM and 59.1 g kg^{-1} DM, respectively (Table 1). This resulted in the highest CP content (131.08 g kg^{-1} DM) and the lowest ADL content (49.14 g kg^{-1} DM) in the FTR diet. No differences among treatments on DM, OM, CP, NDF and ADF intake of the sheep were observed (Table 2). However, apparent digestibility of DM, OM, CP, NDF and ADF were highest ($p\leq 0.014$) in sheep fed the FTR diet and were lowest in sheep fed the RS diet.

Effect of dietary treatment on nitrogen balance: There were no differences in N intake and N excreted in feces and urine among the 3 diets (Table 3). However, the N absorption and N retention were greatest (8.45 and 4.32 g/day, respectively, $p\leq 0.034$) for sheep fed the FTR diet. As a consequence, the N retention as a proportion of the N intake also was highest (35.04%, $p<0.001$) in sheep fed the FTR diet, compared to other diets.

PD excretion and microbial protein supply: Fungal treated rice straw promoted highest (7.17 mmol/day, $p<0.0001$) urinary PD excretion (Table 4), compared to other diets. The estimated microbial N supply was greatest (6.04 g/day,

Table 1: Ingredients and chemical composition of diets containing either rice straw, urea treated rice straw or fungal treated rice straw

	Dietary treatments							
Items	RS	UTS	FTS	Rice straw	Urea treated rice straw	Fungal treated rice straw	Guinea grass	Concentrate
Ingredients (g kg^{-1} DM)								
Rice straw	333.33	-	-	-	-	-	-	-
Urea treated rice straw	-	333.33	-	-	-	-	-	-
Fungal treated rice straw	-		333.33	-	-	-	-	-
*Guinea grass	333.33	333.33	333.33	-	-	-	-	-
**Concentrates	333.33	333.33	333.33	-	-	-	-	-
Chemical composition (g kg^{-1} DM)								
DM (g kg^{-1} basic feed)	449.28	399.70	430.41	892.90	516.50	709.10	223.20	922.40
OM	877.51	878.24	868.81	846.20	848.90	820.30	890.30	895.40
CP	117.50	129.37	131.08	41.90	79.20	83.80	120.90	187.90
NDF	590.34	582.29	532.34	795.10	763.80	616.30	753.60	229.5
ADF	351.93	339.11	325.77	490.90	447.9	409.50	443.80	125.60
ADL	61.38	53.79	49.14	96.30	72.90	59.10	61.10	27.40

DM: Dry matter, OM: Organic matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, RS: Rice straw, UTR: Urea treated rice straw, FTR: Fungal treated rice straw, *Guinea grass: (*Panicum maximum*) was harvested daily at 40-45 days after regrowth during experiment period, **Concentrate: Corn meal, rice bran, wheat bran, soybean meal, palm kernel meal, rapeseed meal, fish meal, premix mineral-vitamin

Table 2: Nutrient intake (g/day) and digestibility (g kg^{-1}) of sheep fed rice straw, urea treated rice straw and fungal treated rice straw

	Dietary treatments				
Parameters	RS	UTR	FTR	SEM	p-value
Nutrient intake (g/day)					
DM	505.47	553.31	586.63	47.220	0.501
OM	443.56	485.95	509.67	41.399	0.543
CP	59.40	71.58	76.89	6.071	0.169
NDF	332.04	380.43	330.50	19.757	0.183
ADF	177.85	187.67	191.12	15.985	0.834
Nutrient digestibility (g kg^{-1})					
DM	598.12^{a}	612.56^{a}	697.95^{b}	9.723	<0.0001
OM	606.36^{a}	628.23^{a}	718.97^{b}	9.645	<0.0001
CP	570.69^{a}	601.69^{a}	687.07^{b}	12.105	0.0002
NDF	563.03^{a}	609.83^{ab}	652.54^{b}	16.837	0.014
ADF	508.91^{a}	539.05^{a}	588.46^{b}	10.553	0.0015

^{a,b,c}Means in the same row with different superscripts differ with p-value mentioned in the Table 2, DM: Dry mater, OMI: Organic matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, RS: Rice straw, UTR: Urea treated rice straw, FTR: Fungal treated rice straw, SEM: Standard error of means

Table 3: Nitrogen balance (g/day) of sheep fed rice straw, urea treated rice straw and fungal treated rice straw

Parameters	Dietary treatments RS	UTR	FTR	SEM	p-value
N intake (g/day)	9.50	11.45	12.30	0.972	0.169
N feces (g/day)	4.07	4.52	3.85	0.325	0.370
N urine (g/day)	3.32	4.39	4.13	0.465	0.283
N absorption (g/day)	5.44^{a}	6.93^{ab}	8.45^{b}	0.671	0.034
N retention (g/day)	2.12^{a}	2.54^{a}	4.32^{b}	0.331	0.0026
As % of N intake					
N retention (%)	22.65^{a}	21.88^{a}	35.04^{b}	1.826	< 0.001

^{a,b,c}Means in the same row with different superscripts differ with p-value mentioned in the Table 3, N: Nitrogen, RS: Rice straw, UTR: Urea treated rice straw, FTR: Fungal treated rice straw, SEM: Standard error of means

Table 4: Purine derivative excretion and microbial N supply of sheep fed rice straw, urea treated rice straw and fungal treated rice straw

Parameters	Dietary treatments RS	UTR	FTR	SEM	p-value
Allantoin (mmol/day)	3.94^{a}	5.08^{b}	5.41^{c}	0.061	<0.0001
Xanthine+hypoxanthine (mmol/day)	0.12^{a}	0.14^{b}	0.15^{b}	0.004	0.0041
Uric acid (mmol/day)	1.42^{a}	1.49^{a}	1.62^{b}	0.020	0.0002
Total purine derivative (mmol/day)	5.48^{a}	6.71^{b}	7.17^{c}	0.079	<0.0001
Microbial N (g/day)	4.47^{a}	5.62^{b}	6.04^{c}	0.069	<0.0001
DOMR (kg/day)	0.18	0.20	0.24	0.019	0.110
EMNS (g microbial N/kg DOMR)	25.80	29.77	25.62	2.553	0.461

^{a,b,c}Means in the same row with different superscripts differ with p-value mentioned in the Table 4, N: Nitrogen, DOMR: Digestible organic matter fermented in the rumen, EMNS: Efficiency of microbial N synthesis, RS: Rice straw, UTR: Urea treated rice straw, FTR: Fungal treated rice straw, SEM: Standard error of means

p<0.0001) in the FTR diet. However, the efficiency of microbial N synthesis (EMNS), expressed on the basis of digestible OM intake was not significantly different among diet treatments.

DISCUSSION

El-Bordeny *et al.*[18] reported that DM intake was similar when the growing lambs fed fungal (*Trichoderma reesei* and *Trichoderma viride*) treated rice straw, compared to untreated rice straw. Fazaeli *et al.*[8] also found that DM, OM and CP intake were not different by replacing alfalfa hay with fungal (*Pleurotus*) treated wheat straw in dairy cows diets. However, in another study, the authors found that DM intake was greater (321.75 vs. 297.5 g/head/day, p<0.05) in goats fed fungal (*Ganoderma* sp. rckk02) wheat straw, compared to goats fed untreated straw[19].

The *in vitro* DM and OM digestibility increased from 67.11 and 56.16%, respectively in untreated rice straw to above 70% in fungal (*Pleurotus*) treated rice straw[6]. Increasing *in vitro* OM digestibility was also detected in fungal treated rice straw, compared to untreated rice straw in the study of Akinfemi and Ogunwole[5]. The results of the current study also agreed with the finding of Omer *et al.*[20] who found that DM, OM, CP and CF digestibility increased when replacing clover hay by fungal (*Trichoderma ressi*) treated corn stalks in growing sheep rations. Nutrient digestibility was highest (p<0.019) in sheep fed fungal (*Trichoderma viride*) peanut hulls, compared to sheep fed urea treated peanut hulls and untreated peanut hulls[21]. Lignin content is one of major barriers preventing micro-organism enzymes to digest nutrients in rice straw[22]. However, fungi can delignify the rice straw and this results in removal of lignin and a subsequent opening of structural fiber. Therefore, this could lead to an increase in rice straw nutrient digestibility[23]. In the current study, the ADL content of fungal treated rice straw was lower (59.1 g kg^{-1} DM) than in the untreated rice straw (96.3 g kg^{-1} DM). As a consequence, the ADL content was lowest in the FTR diet. Saxena *et al.*[24] found that administration of anaerobic fungi in the rumen of domestic ruminants resulted in increasing nutrient digestibility of the diets. Therefore, in the current study, when rice straw was treated with fungus, nutrient value of rice straw was improved and lignin content of rice straw was reduced. The mycelium of fungus in the fungal treated rice straw diet might regrow in the rumen and combine with the ruminal fungi and secrete more enzymes to digest other feeds in the rumen. This could be a reason that the highest nutrient digestibility were detected in sheep fed FTR diet.

Improving N retention (3.74 g/day) also found in goats fed fungal treated wheat straw[19]. The higher N retention (p = 0.023) also found in sheep fed fungal (*Trichoderma viride*) peanut hulls, compared to sheep fed urea treated peanut hulls and untreated peanut hulls[21]. However, El-Bordeny *et al.*[18] found that N retention was not significantly different between growing lambs fed fungal treated wheat straw and untreated straw. The highest N retention found in FTR diet might be explained by the fungal treatment increases protein content and the protein digestibility. Also, energy supply in these sheep might be higher because fungi can delignify the rice straw and subsequently the structural carbohydrate of the cell wall becomes more accessible to micro-organism in the rumen[25].

Orden *et al.*[26], who reported the higher PD excretion, the higher Microbial Protein Synthesis (MPS) in the diet. The higher in microbial protein synthesis also found in *in vitro* ruminal fermentation when wheat straw treated with *Polyporus ciliatus*, compared to untreated wheat straw[27]. The PD excretion is affected either by changes in outflow rate from the rumen or by the intestinal flow of MPS. Fungal treated rice straw improved nutrients digestibility and provided more degradable organic matter in the rumen, therefore, it could result in faster outflow rates and could lead to higher MPS. Abo-Donia *et al.*[21] stated that increasing microbial protein synthesis resulting in a higher N retention. The above results suggested that fungal treated rice straw could be a potential feed for ruminants to meet the requirements of organic agriculture and friendly environment.

SIGNIFICANCE STATEMENT

This study discovers the used of fungal treated rice straw in the sheep diet increased nutrient digestibility and microbial protein synthesis. Therefore, the study would be pointed out that fungal treated rice straw could be a potential ruminant feed to meet the requirements of organic agriculture and friendly environment.

CONCLUSION

Using fungal treated rice straw in the diet of sheep showed the best improvement of nutrient digestibility, N retention and microbial protein synthesis without adverse effect on growth performance indicating that besides urea treated rice straw, *Pleurotus eryngii* treated rice straw have a good potential as ruminants feed resources in Vietnam.

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