Article

Treatment of rice stubble with *Pleurotus ostreatus* and urea improves the growth performance in slow-growing goats

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Simple Summary: Fungi treatment is well-established as a promising approach to upgrade the nutritional value of lignocellulosic biomass. This potency of fungi treatment is, however, primarily based on *in vitro* experiments and extrapolation to practice is currently hindered owing to a dearth of studies addressing the practical relevance of fungal treatment of high fiber feed, such as rice straw and rice stubble. These potential biomasses are rife in Southeast Asian countries coinciding with increasing rice production; however, it remains a big challenge to utilize rice stubble as a potential feed for ruminant. Similar to rice straw, rice stubble is traditionally eliminated through controlled burning, which is harmful to the environment. The aim of this study was to convert rice stubble into a new animal feed capable of increasing environmental-friendly. Using urea, it is a well-known to modify lignification or silicification of lignocellulosic biomass. However, it remains scanty in combination with fungi treatment. Therefore, we treated rice stubble with either urea or oyster fungus (*Pleurotus ostreatus*) or a combination of these two treatments and offered these treated rice stubbles to slow-growing goats with the objective to study their effect on feed intake, digestibility, and fermentation end-products.

Abstract: The objective of this study was to evaluate the efficacy of fungal treatment (*Pleurotus ostreatus*) of urea-treated rice stubble on growth performance in slow-growing goats. Eighteen crossbred Thai native x Anglo-Nubian male goats (average body weight: 20.4 ± 2.0 kg) were randomly assigned to three experimental total mixed rations containing 35% rice stubble (RS) that was either untreated (URS), urea treated (UTRS) or treated with urea and fungi (UFTRS). URS and UTRS were cultivated and harvested from aseptically fungal spawn, incubated at 25-30 °C for 25 days. Indicators of growth performance were monitored and faeces were collected quantitatively to assess nutrient digestibility, during a 12-week feeding trial. All goats remained healthy throughout experiment. The goats fed UFTRS had a lower feed conversion ratio (kg feed/kg growth) compared to goats fed URS or UTRS. Compared to URS, dietary UFTRS increased nutrient digestibility of slow-growing goats, such as OM (+8.5%), CP (+5.5%), NDF (+39.2%), and ADF (+27.4%). Likewise, dietary UFTRS tended to increase rumen ammonia concentrations but rumen pH and volatile fatty acids were not affected by UFTRS. In conclusion, the present study indicates that the fungal treatment of RS is an effective tool to improve the growth performance of slow-growing goats.

1. Introduction

Within the Southeast Asian countries, Thailand is a large producer of rice and thus hugh amounts of biomass, in the form of rice straw and rice stubble, are available for the feeding of ruminants [1]. Those agricultural coproducts provides a source of roughage typically relevant in times of feed scarcity such as the dry season [2]. Despite being poorly fermented, and slow disappearance from the rumen, rice straw can be used as an alternative forage source for ruminants after following processing the original biomass. For instance, calcium hydroxide or urea have been used for modifying either the lignification or silicification of rice straw and such treatments results in an increased dry matter intake and milk production [3]. These benefit values of rice straw utilization have been a gradually mount interest by researchers in enhancing feed value for ruminant [2]. In case of rice straw scarcity, rice stubble can also be considered as a roughage source. The nutritional value of rice stubble is however, poor mainly because of its high fibre content [4]. Therefore, processing of rice stubble to increase its nutritional value can be considered opportune.

Of interest, few previously studies [5-7] indicated that the treatment of lignocellulosic biomass with white-rot fungi is one of promising tools to modulate the nutritional value of highly lignified material. The potential of various white-rot fungi species, including *Pleurotus ostreatus*, to increase the nutritional value of tough lignified material is primarily based on *in vitro* studies [5,7,8], whereas *in vivo* studies are needed to demonstrate the efficacy of fungal treatment of lignocellulosic biomass under feeding conditions. However, extrapolation of the current *in vitro* results to practice is currently hindered owing to a dearth of studies addressing the practical relevance of fungal treatment of high fibre feed such as rice stubble.

Therefore, the aim of present study was to evaluate the efficacy of fungal treatment (*Pleurotus ostreatus*) of urea-treated rice stubble on growth performance of slow-growing goats and it was hypothesized that the treatment of rice stubble with *Pleurotus ostreatus* increases the efficiency of feed conversion to body weight gain in slow-growing goats. To date, treatment of rice straw with urea is generally practiced to increase its digestibility [3,4]. This method is perceived as easy, reproducible, and relatively cheap approach for further potential to increase the digestibility of rice straw, where it also increases the N content of the feed. The latter is considered relevant due to the nitrogen content of rice straw is generally less than 2% on dry matter (DM) basis [9], and thus limits rumen fermentation [10,11]. As consequence, it was considered opportune to evaluate the efficacy of *Pleurotus ostreatus* on growth performance using urea treated rice stubble as a control. A dietary treatment containing untreated rice stubble was used as a negative control.

2. Materials and Methods

2.1. Ethical statement

The research was carried out in accordance with regulations on animal experimentation, and the guidelines for the use of animals in research as recommended by the National Research Council of Thailand (U1-02632-2559). The Animal Ethics Committee of Suranaree University of Technology issued a statement approving the experimental protocol (SUT 4/2558).

2.2. Preparation of Treated Rice Stubble

Rice stubble (RS) was collected and sampled from random spots at paddy field near SUT Organic Farm, Nakhon Ratchasima, Thailand (14°52′48′′N, 102°00′54′′E at an elevation of 243 m above sea level). Rice material which remaining in RS was removed gently by hand. RS then was mechanically chopped into particles of 2-5 cm length. Thereafter, ~300 kg of boiling water was added to each 100 kg of RS and the moisturized RS was left overnight so as to enable water to penetrate into the inner structures of the RS and to minimize contamination by unwanted fungal spores.

The RS intended for the urea-fungi-treated RS (UFTRS) treatment, was then transferred into plastic bags and inoculated with previously prepared spawn of *Pleurotus ostreatus* as described by Vorlaphim et al. [12] and the plastic bags with inoculated RS were incubated at ambient temperature (25-30 °C) for 25 days. At day 21 of the incubation period, urea was added (2.5 %) to terminate the fungal activity. The RS intended for the urea-treated RS (UTRS) treatment was prepared almost similar to that of the UFTRS treatment; i.e., 2.0% instead of 2.5% urea was added to the moisturized rice stubble and, for obvious reasons, the RS was not inoculated with *Pleurotus ostreatus*. The aforementioned difference in urea treatment was deemed necessary to attain iso-nitrogenous rations. The chemical composition of untreated RS (URS), UTRS, and UFTRS is shown in Table 1.

Table 1. Chemical composition of untreated rice stubble (URS), urea-treated rice stubble (UTRS), and urea-fungi-treated rice stubble (UFTRS).

Item	URS	UTRS	UFTRS
Dry matter (g/kg fresh weight)	960	239	230
Organic matter (g/kg DM)	849	793	771
Crude protein (g/kg DM)	25	41	95
Ether extract (g/kg DM)	9	8	8
Neutral detergent fiber (g/kg DM)	779	768	659
Acid detergent fiber (g/kg DM)	580	597	591
Acid detergent lignin (g/kg DM)	49	40	35

2.3. Animals, Treatments, and Experimental Design

Eighteen, crossbred Thai native x Anglo Nubian male goats with an initial average body weight of 20.4 kg (SD 2.0 kg, *n*=18) were used. These typically slow-growing goats were obtained from the university farm of Suranaree University of Technology, Thailand. The experiment had a parallel design and lasted for 12 weeks. The goats were randomly assigned to their, iso-nitrogenous, experimental rations; i.e., a total mixed ration (TMR) consisting of 35% rice stubble (RS) that was either untreated RS (URS), or urea-treated RS (UTRS), or urea-fungi-treated RS (UFTRS, Table 2).

The experiment was preceded by a 14-day adaption period, during which the animals could become accustomed to the RS-containing rations. Animal health management was following to our previous study [13]. The goats were housed in individual metabolic cages (length 2.2 m × width 1.3 m × height 2 m) and the experimental rations were offered *ad libitum*, twice daily at 0700 and 1700 h. Feed refusals were recorded each 24-h period so

as to calculate daily feed intake. Fresh drinking water was available at all times. Each animal was weighed on the last day of the adaption period and on days 21, 49 and 77 of feeding trial.

Table 2. Ingredient and chemical composition of total mixed ration (TMR) diets consisting of untreated rice stubble (URS), urea-treated rice stubble (UTRS), and urea-fungi-treated rice stubble (UFTRS).

Item	TMR diet		
	URS	UTRS	UFTRS
Ingredient, g/kg DM			
Constant components[1]	150	150	150
Cassava hay	470	485	493
Untreated rice stubble	350	-	-
Urea-treated rice stubble	-	350	-
Urea-fungi-treated rice stubble	-	-	350
Urea	30	15	7
Chemical composition, g/kg DM			
Dry matter (g/kg fresh weight)	463	488	420
Organic matter (g/kg DM)	918	922	845
Crude protein (g/kg DM)	122	120	123
Ether extract (g/kg DM)	15	16	16
Neutral detergent fiber (g/kg DM)	354	354	325
Acid detergent fiber (g/kg DM)	244	254	241
Acid detergent lignin (g/kg DM)	17	3	03
Non-structural carbohydrates[2]	427	432	382

[1] The constant components consisted of the following ingredients (% as fed): rice bran, 5.0; soybean meal, 3.0; molasses, 5.0, salt, 0.4; sulfur, 0.2; di-calcium phosphate, 0.5, limestone, 0.2, premix, 0.7.
[2] Calculated as organic matter - crude protein - ether extract - neutral detergent fiber.

2.4. Sample Collection

Feed was sampled on the following days of the feeding trial; from day 22 to day 28, from day 50 to day 56 and from day 78 to day 84. The daily samples were divided into two parts, the first part was analysed for DM and the second part was kept and pooled at the end of each sampling period. The pooled feed samples were then dried for 72 hours at 60 °C, subsequently ground (1 mm screen) by Cutting Mill (Retsch SM 100 mill; Retsch Gmbh, Haan, Germany) and stored in a sealed jar at ambient temperature until analysis.

During the aforementioned periods, faeces were collected quantitatively from each goat. Each 24-h collection of faeces was weighed and ~5% of each collection was dried for 72 hours at 60 °C. The dried samples were stored in plastic bags at ambient temperature (25-30 °C) pending analysis.

Rumen fluid was collected (~ 500 mL) on the next day following the period of faeces collection with the use of a stomach tube connected to a manual pump [14-16]. The rumen fluid samples were taken ~ 30 minutes before the morning meal and 2 and 4 hours after the morning feeding. Immediately after collection, pH of the rumen fluid samples was recorded and the rumen fluid samples were filtered through four layers of cheesecloth. Then, 10 mL of a 50% H_2SO_4 solution was added to 100 mL of filtered rumen fluid and the mixture was subsequently centrifuged at 16,000 x g for 15 minutes. The supernatant was stored at -20 °C until the analysis of ammonia and volatile fatty acids.

2.4. Chemical Analysis

Prior to the chemical analysis of faeces, the samples were pooled per goat for each collection period and subsequently ground (1 mm screen). Feed and faeces were analysed for analysis of crude ash, ether extract (EE) and crude protein (CP) contents using the standard procedures as described by the AOAC [17]. Crude protein was calculated as N x 6.25. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using the standard procedures method as described by Van Soest et al. [18].

Hemicellulose was calculated as NDF minus ADF. Ammonia contents of rumen fluid were determined according to Bremner and Keeney [19] and volatile fatty acids were measured by means of HPLC (RF-10AXmugiL; Shimadzu; Japan) as described by Zinn Zinn and Owens [20] with minor modification in peak analyses as given by Purba and Paengkoum [21].

2.5. Stastical Analysis

The efficacy of fungal treatment (*Pleurotus ostreatus*) of urea-treated rice stubble on indices of growth performance including nutrient intake, nutrient digestibility, and its ruminal fermentation end-product was subjected to ANOVA using the MIXED procedure of SAS 9.4.

The statistical model used was:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, \tag{1}$$

where Y_{ij} = response variable, μ = overall mean, τ_i = effect of treatment ($i = 3$) and ε_{ij} = residual error. Tukey HSD test was used to identify rations with different effects on the variable involved. Throughout, the level of statistical significance was pre-set at $P < 0.05$.

3. Results

3.1. Growth Performance

Initial body weight (BW) of the goats (Table 3) allocated to total mixed ration (TMR) containing only RS or untreated RS (URS), and urea-treated RS (UTRS) were similar, but the initial BW of the goats allocated to urea-fungi-treated RS (UFTRS) were lower ($p < 0.05$) compared to the goats treated either with URS (7.8%) or UTRS (14.1%). Despite the difference in initial BW, final BW of the goats was found to be similar ($p > 0.05$) among those experimental diets after those goats had a 12-week feeding trial. Consequently, the highest BW gain and growth rate were found in the goats fed UFTRS, and both values were ~ 1.8 times greater ($p < 0.05$) than the corresponding values observed in the goats fed URS. Daily feed intakes were similar between URS and UTRS; however, the goats fed UFTRS consumed 11.1% more feed ($p < 0.05$) that the goats fed URS. Nevertheless, the feed conversion ratio (Table 3) of the animals fed UFTRS was found to be 39.1% lower ($p < 0.05$) compared to the goats fed URS, but UTRS did not affect the on-feed conversion ratio in the slow-growing goats ($p > 0.05$).

Table 3. Selected indices of growth performance of slow-growing goats after the feeding of total mixed ration (TMR) diets consisting of untreated rice stubble (URS), urea-treated rice stubble (UTRS), and urea-fungi-treated rice stubble (UFTRS).

Item	TMR diet			SEM	*p* Value
	URS	UTRS	UFTRS		
Body weight (kg)					
Initial	20.5[ab]	22.0[a]	18.9[b]	0.68	0.022
Final	23.2	25.0	23.9	0.74	0.226
Gain	2.7[b]	3.1[ab]	5.0[a]	0.58	0.036

Growth rate (g/d)	35.6[b]	39.6[ab]	64.9[a]	8.25	0.036
Feed intake (g DM/d)	618.5[b]	658.5[b]	687.4[a]	6.62	0.003
Feed conversion ratio (g/g)[1]	17.4[a]	16.6[a]	10.6[b]	1.39	0.015

[ab] Means in the same row with a different superscript, differ significantly ($p < 0.05$). [1] Calculated as g of feed / g of body weight gain. SEM: standard error of mean.

3.2. Macronutrient Intake and Digestibility

The intake of organic matter (OM) was similar between URS and UFTRS ($p > 0.05$) and the highest OM intake was observed when the goats were fed UTRS ($p < 0.05$). In contrast, the highest value on OM digestibility was found when the goats were fed UFTRS; the value being 8.5% greater ($p < 0.05$) compared to UTRS. Crude protein (CP) intakes were mirrored by the DM intakes and the highest value on CP digestibility was found when the goats were fed UFTRS. The intakes of neutral- and acid detergent fibre (NDF and ADF, respectively) were significantly higher when the goats were fed UTRS, but respective intakes were similar ($p > 0.05$) to those when UFTRS was fed. The feeding of UFTRS, but not URS and UTRS, resulted in significantly higher values on NDF and ADF digestibility. Compared to URS, the respective NDF and ADF digestibility values were 39.2% and 27.4% higher ($P < 0.05$) when UFTRS was fed to the goats. The intake of hemicellulose was lowest ($P < 0.05$) when goats were fed UFTRS, but its digestibility was almost two times greater ($P < 0.05$) compared to UTRS. The goats fed UTRS had the highest intakes ($P < 0.05$) of non-structural carbohydrates (NSC), but NSC digestibility was only affected ($P < 0.05$) when UFTRS was fed (Table 4).

Table 4. Mean macronutrient intake and apparent fecal digestibility after slow-growing goats fed total mixed ration (TMR) diets consisting of untreated rice stubble (URS), urea-treated rice stubble (UTRS), and urea-fungi-treated rice stubble (UFTRS). The values represent the overall means obtained during three distinct periods of the 12-week feeding trial.

Item	TMR diet			SEM	*p* Value
	URS	UTRS	UFTRS		
Organic matter					
Intake (g/d)	567.9[b]	607.0[a]	580.8[ab]	5.77	0.041
Digestibility (% of intake)	62.6[b]	65.9[b]	71.5[a]	1.02	0.010
Crude protein					
Intake (g/d)	75.5[b]	79.2[b]	84.7[a]	0.81	0.001
Digestibility (% of intake)	57.6[b]	63.6[ab]	67.1[a]	1.51	0.050
Neutral detergent fiber					
Intake (g/d)	218.9[b]	233.2[a]	223.0[ab]	2.22	0.050
Digestibility (% of intake)	46.9[b]	53.6[ab]	65.3[a]	2.31	0.017
Acid detergent fiber					
Intake (g/d)	150.9[b]	167.1[a]	165.3[a]	1.61	0.002
Digestibility (% of intake)	44.9[b]	51.1[ab]	57.2[a]	2.05	0.018
Hemicellulose[1]					
Intake (g/d)	67.9[a]	66.1[a]	57.7[b]	0.66	<0.001
Digestibility (% of intake)	35.1[b]	42.5[b]	69.1[a]	3.08	<0.001
Non-structural carbohydrates[2]					
Intake (g/d)	264.2[b]	284.1[a]	262.3[b]	2.64	<0.001
Digestibility (% of intake)	78.1[b]	80.6[b]	82.4[a]	0.23	0.020

[ab] Means in the same row with a different superscript, differ significantly ($p < 0.05$). [1] Calculated as neutral detergent fiber - acid detergent fiber. [2] Calculated as organic matter - crude protein - ether extract - neutral detergent fiber. SEM: standard error of mean

3.3. Ruminal Fermentation End-Product

Rumen pH and total volatile fatty acids (VFA) were similar ($p > 0.05$) among the dietary treatments (URS vs. UTRS vs. UFTRS, Table 5). For the three dietary treatments, three measurement days, and the three time points/day combined, the overall mean values on rumen pH value and total VFA concentrations were found to be 6.69 (SD 0.05, n=3) and 87.0 mM (SD 3.0, n=3), respectively. The proportion of acetic acid (% of total VFA), was similar ($p > 0.05$) among the treatments both before (0 h), and 4 h after the morning feeding, i.e., 59.2% (SD 0.21, n=3) and 59.3% (SD 0.61, n=3). However, UFTRS was tendency for greater proportion of acetic acid at 2 h post-feeding, which the values were 58.9, 61.3 and 66.1% of total VFA for URS, UTRS and UFTRS, respectively. In contrast to acetic acid, the proportions of propionic- and butyric acid were not influenced by dietary treatments. For the three dietary treatments, three measurement days, and the three time points/day combined, the overall mean values on the proportions of propionic- and butyric acid (% of total VFA) were found to be 28.1% (SD 0.81, n=3) and 7.7% (SD 0.77, n=3), respectively.

Rumen ammonia concentration was greater ($p < 0.05$, Table 5) when the goats were fed UFTRS. For the three measurement days and the three time points/day combined, the mean rumen ammonia values were found to be 15.5, 15.9 and 18.3 mg/100 mL after the feeding of URS, UTRS and UFTRS, respectively.

Table 5. Fermentation end-product in rumen fluids of slow-growing goats after the feeding of total mixed ration (TMR) diets consisting of untreated rice stubble (URS), urea-treated rice stubble (UTRS), and urea-fungi-treated rice stubble (UFTRS).

Item	TMR diet			SEM	p Value
	URS	UTRS	UFTRS		
Ruminal pH					
0 h post-feeding	7.09	6.82	6.94	0.05	0.120
2 h post feeding	6.78	6.59	6.58	0.06	0.290
4 h post feeding	6.34	6.53	6.52	0.05	0.250
Mean	6.74	6.65	6.68	0.03	0.450
NH$_3$-N concentration, mg/dL					
0 h post-feeding	13.00	14.21	16.15	0.53	0.080
2 h post feeding	16.31[b]	17.38[b]	20.72[a]	0.42	0.002
4 h post feeding	17.19[ab]	16.12[b]	18.01[a]	0.22	0.010
Mean	15.50[b]	15.90[b]	18.29[a]	0.29	0.002
Total VFA, Mm					
0 h post-feeding	75.36	62.92	75.20	4.16	0.400
2 h post feeding	92.66	92.02	84.60	3.23	0.540
4 h post feeding	98.79	110.57	90.81	5.34	0.340
Mean	88.93	88.50	83.54	1.06	0.600
VFA profile, mol/100 mol					
Acetic acid (C2)					
0 h post-feeding	58.99	59.41	59.25	0.93	0.98
2 h post feeding	58.85[b]	61.34[ab]	66.14[a]	0.07	0.02
4 h post feeding	58.94	58.84	59.94	0.75	0.80
Mean	58.93	59.87	61.78	0.46	0.07
Propionic acid (C3)					
0 h post-feeding	30.82	28.64	30.40	0.95	0.62
2 h post feeding	27.78	28.00	24.05	0.79	0.10
4 h post feeding	28.34	27.13	27.75	0.85	0.85
Mean	28.98	27.92	27.40	0.36	0.23
Butyric acid					
0 h post-feeding	10.19	11.95	10.35	0.49	0.30
2 h post feeding	13.36	10.65	9.80	0.64	0.09

4 h post feeding	12.72	14.03	12.31	0.53	0.40
Mean	12.09	12.21	10.82	0.28	0.12
C2:C3 ratio					
0 h post-feeding	1.97	2.13	1.97	0.10	0.75
2 h post feeding	2.16[b]	2.22[b]	2.81[a]	0.11	0.04
4 h post feeding	2.14	2.23	2.18	0.10	0.94
Mean	2.09	2.20	2.32	0.04	0.14

[ab] Means in the same row with a different superscript, differ significantly ($p < 0.05$). NH$_3$-N: ammonia. VFA: volatile fatty acid. SEM: standard error of mean

4. Discussion

To the best of the author's knowledge, the current data show for the first time that fungal treatment of urea-treated rice stubble improves the feed conversion efficiency in slow-growing goats. This result is in line with the observation that the fungal treatment of rice stubble caused a significantly greater OM digestibly, irrespective the urea treatment of rice stubble (Table 4). This notion is substantiated by the fact that, at least numerically, OM intake was lowest when the goats were fed UFTRS. It thus appears that the previous *in vitro* data reported on fungi induced improvement of OMD [5,7,8] can be extrapolated, at least qualitatively, to practical feeding conditions.

Fungal treatment of UFTRS caused a greater DM but not OM intake compared to URS and URS. This observation could be explained by the lower OM content of the UFTRS ration (Table 1). In the current study, UFTRS versus UTRS did not stimulate OM intake. Unfortunately, there are currently no reports available to substantiate this observation. The lack of effect of UFTRS on OM intake is however not easy to explain because it does not seem to be in line with the observed increases in apparent NDF and ADF digestibility's when UFTRS was fed. Indeed, the dietary content of rumen digestible NDF is generally believed to be the main determinant of rumen fill and therefore of DM intake in ruminants with unrestricted access to roughage-based rations [22]. It, thus, can be speculated that the disappearance rate of NDF from the rumen was not affected by the fungal treatment of UFTRS. This notion is in line with the current observation that the feeding of UFTRS did neither affect rumen pH nor total VFA concentrations. Thus, the current observations do not support the idea that rumen fermentation is stimulated by UFTRS. It is therefore hypothesized that the UFTRS induced increase in total tract digestibility of NDF, is explained by an increased hindgut fermentation of structural carbohydrates. Needless to say, that the current study does not provide further clues to support this idea and future studies are required to test this hypothesis.

In general, white-rod fungi are the only fungi capable to degrade lignin in its substructures [23], such as guaiacyl-, syringyl- and *p*-hydroxyphenyl units [24]. In the process of lignin degradation, cellulose, and hemicellulose are liberated and subsequently attacked by fungal glycoside hydroxylases [23], thereby yielding substrate for microbes and thus potentially improving the nutritional value of lignocellulosic biomass. In the present study, *Pleurotus ostreatu*s was used to treat UFTRS. However, it should be taken into consideration that the cause-and-effect relationship between fungal treatment and improvement of the nutritional value of lignocellulosic biomass is not straightforward due to a great variety in both fungal species and chemical background of the available lignocellulosic biomass [6]. Clearly, the current results do not preclude the possibility that white-rode species and/or varieties other than *Pleurotus ostreatu*s, are at least equally effective in increasing apparent faecal digestibility. Indeed, it is well known that *Pleurotus eryngii* as well as certain *Ceriporiopsis subvermisopra* varieties have great potential to effectively degrade lignocellulosic biomass [7].

In contrast to expectation [3,4], urea treatment of rice stubble did not significantly affect OM digestibility. There was however a numerical increase of OM digestibility of 5.3%. The observed increase in OM digestibility is in line with data reported by Zhang et

al. [25] who reported an increase of 3.3 % in OM digestibility in goats fed urea treated instead, of untreated, rice straw. Despite the lower impact of urea treatment on the OM digestibility, the latter result of Zhang's observation was found to be statistically significant. The difference in probability values of type I error between the two studies is, at least partially, most likely caused by the number of animals used, i.e., 9 [25] versus 6 (current study) goats per treatment because the corresponding SEM values are almost identical, i.e. 1.05 and 1.02, respectively.

The fungal treatment of UFTRS caused greater rumen ammonia concentrations but the explanation for this observation is unfortunately not straightforward due to the fact that N intakes were ultimately somewhat greater when the goats were fed UFTRS. On the other hand, it may be suggested that the fungal treatment of rice stubble rendered more protein available for rumen fermentation. This suggestion is corroborated by data obtained from *in vitro* research conducted by Nayan et al. [26] showing that fungal treatment of wheat straw with two different strains of *Pleurotus eryngii* increased the NPN fraction in buffered rumen fluid under *in vitro* conditions. Moreover, the increase in NPN was associated with a decrease in cell wall bound proteins. Perhaps, *Pleurotus ostreatus* also is able to liberate proteins bound to cell walls thereby explaining, at least partly, the greater rumen ammonia concentrations after the feeding of UFTRS. The latter notion may also explain the observed greater apparent digestibility of CP when the goats were fed the fungi treated rice stubble.

5. Conclusions

It is concluded that the fungal treatment of urea treated rice stubble is a practical tool to improve the feed conversion ratio in slow-growing goats. The improved growth performance is caused by an improved total tract nutrient digestibility. Further studies are however warranted to understand the *in vivo* cause-and-effect relationship between the fungal treatment of rice stubble and total tract digestibility of structural carbohydrates in particular**.**

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