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Using of Bio-Upgraded Rice Straw in Growing Lambs Nutrition

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ABSTRACT

The objective of this study was to investigate the ability of biological treatment (*Trichoderma viride* and *Trichoderma reesei* application) of rice straw to improve its chemical composition and cell wall constituents, as well as to evaluate male lamb's performance fed ration containing treated rice straw. Thirty crossbred (Rahmany X Romanof) lambs (3 months old, 21±1.46 kg body weight) were randomly assigned to three groups (10 lambs each). Group one was fed ration contained 57% concentrate feed mixture and 19% clover hay plus 24 Untreated Rice Straw (URS), while group two and three were fed rations containing treated rice straw with *Trichoderma reesei* (TRSV) and *Trichoderma viride* (TRSR) instead of URS, respectively. Both of biologically treated rice straw TRSV and TRSR recorded lower OM, NDF, ADF and cellulose contents sthan URS. While TRSV and TRSR recorded higher CP, Ash and ADL contents compared to URS. Recovery rate as DM and OM for TRSV were higher than those for TRSR which was 88.05 vs 85.01 for DM recovery and 86.17 vs 83.47 for OM recovery. Insignificant differences were observed in nutrients digestibility, while insignificant increase in nutritive values as digestible protein and total digestible nutrients as well as nitrogen retention were observed for groups fed TRSV and TRSR compared to control. No significant differences were observed in rumen liquor TVFA's, ammonia-N concentration and pH value among groups at 0, 3 and 6 h after feeding. Groups received TRSV and TRSR were significantly higher total gain and average daily gain and recorded better feed conversion as DM and CP compared to the control group. It could be concluded that biodegradation of rice straw using *T. viride* and *T. reesei* upgrade its chemical composition and inclusion TRSV and TRSR in animal ration had no adverse effect on animal performance.

Key words: Biological treatment, digestibility, nitrogen balance, gain

INTRODUCTION

There is a wide gap between the available feeds and animals requirements in Egypt. It was estimated by about 6 million tons in term of concentrate feeds and as a shortage of 4.79 million tons of TDN per year (El-Ashry, 2007). In addition, accumulation of agriculture crop residuals especially rice straw has considered one of the important reasons of environmental problems during summer season which residues are burned or wasted and hence lead to environmental pollution and health hazards. Only 4.15 million tons of crop residues out of 33.477 million tons produced are used for feeding ruminants (Ministry of Agriculture, 2006). Not only these by-products can be used in favor of solving feed shortage problem but also as a method to control environmental pollution (El-Ashry *et al.*, 2003; Khattab *et al.*, 2009). The major limitation of using rice straw as animal feed

is their lower nutritive values which mainly due to the linkage between polysaccharide and lignin (lignocelluloses bond) are not available to animal digestive hydrolysis, low protein content, high fiber content and low degradability (Van Soest, 2006). Consequently treatments of these materials become essential in order to degrade lingo-cellulosics compounds into lignin, cellulose and hemicellulose and improve crude protein content. During the last years, many efforts have been employed to remove the lignin and/or to break up the linkages between lignin and carbohydrates and to increase their feeding values by biological treatments (Karunanadaa and Varga, 1996; Liu and Orskov, 2000; Zhu *et al.*, 2005; Eun *et al.*, 2006). Therefore, the objective of this study was to investigate the ability of biological treatment (*Trichoderma viride* and *Trichoderma reesei* application) of rice straw to improve its chemical composition and cell wall constituents, as well as to evaluate performance of growing lamb fed ration containing treated rice straw.

MATERIALS AND METHODS

This study was carried out at Mahallet Mossa, farm station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Research Laboratories of Animal Nutrition, Department of Animal Production, Faculty of Agriculture, Ain Shams University.

Preparing of the microorganisms and biological treatment

Microorganisms: *Trichoderma reesei* and *Trichoderma viride* were obtained from Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Minufiya University. *Trichoderma reesei* and *T. viride* were maintained on Potato Dextrose Agar (PDA) medium at 25°C until used. Spore suspension of *T. reesei* and *T. viride* were prepared and used to inoculate a sterilized liquid medium containing 40 g molasses, 4 g urea, 2 g KH₂ PO₄ and 0.3 g MgSO₄.7H₂O/per Liter distilled water and incubated for 7 days at 25°C.

Laboratory treatments: The fungal biomass including the inoculated medium was used to inoculate the polyethylene bags containing 50 g of sterilized rice straw, with 60% moisture level and incubated at 28°C by Solid State Fermentation System (SSFS) for 3 weeks. At the end of the incubation period, the bags were opened and its contents were oven dried at 65°C for 24 h and milled, then subjected to chemical analysis. The DM and OM recovery were calculated according the following formula:

$$\text{Recovery rate} = \frac{\text{Recovered matter weight}}{\text{Initial matter weight}} \times 100$$

$$\text{While nutrient degradation} = \frac{\text{Nutrient initial weight} - \text{Nutrient recovered weight}}{\text{Nutrient initial weight}} \times 100$$

Field treatments: The fungal biomass including the inoculated medium was used to inoculate rice straw which 2 L culture medium for *T. reesei* and *T. viride* separately were added to 500 L of tap water and mixed well with 400 kg rice straw (to get 60% moisture in the mixture) and maintained for 21 days under air temperature in shaded place with daily mixing. Then dried in sunny place and stored until used in animal rations.

Growth trial: Thirty crossbred (Rahmany X Romanof) lambs (3 months old, 21±1.46 kg body weight) were randomly assigned to three groups, 10 lambs each according to live body weight. Each

Table 1: Ingredients and chemical composition of the three experimental total mixed rations (g kg⁻¹)

Item	Biological treatments			Experimental rations		
	URS	<i>T. viride</i>	<i>T. reesei</i>	R1	R2	R3
Carboxymethyle cellulase						
(CMCase) activity		532.7	9003.2			
Ingredient (%)						
Concentrate feed mixture				566	566	566
Clover hay				191	191	191
Untreated rice straw				243	0.00	0.00
Treated rice straw (viride)				0.00	243	0.00
Treated rice straw (reesei)				0.00	0.00	243
Constituents						
Organic matter (OM)	822.4	804.8	807.5	888.5	884.2	884.9
Ash	177.6	195.2	192.5	111.5	115.8	115.1
Crude protein (CP)	53.0	101.6	68.1	126.5	138.3	130.2
Ether extract (EE)	16.6	19.5	18.5	30.8	31.6	31.3
Cell wall constituents						
Neutral-detergent fiber (NDF)	687.4	634.5	651.9	529.1	516.2	520.4
Acid- detergent fiber (ADF)	508.3	497.0	478.8	359.6	356.9	352.4
Acid-detergent lignin (ADL)	83.7	105.3	104.5	72.7	77.9	77.7
Hemicellulose	179.1	137.5	173.1	169.4	159.3	168.0
Cellulose	424.6	391.7	374.3	287.0	279.0	274.7

URS is untreated rice straw, TRSV is rice straw treated with *Trichoderma viride*, TRSR is rice straw treated with *Trichoderma reesei*, CFM is Concentrate feed mixture

group was assigned randomly to receive one of the three experimental rations. The experimental rations were formulated to cover lambs allowances according to NRC (1985). The first ration (control) contained 56.62% concentrate feed mixture and 19.05% clover hay plus 24.33 untreated rice straw (URS), while treated rice straw with *Trichoderma reesei* (TRSV) and *Trichoderma viride* (TRSR) were replaced by URS in the second and third rations, respectively. Formulation and chemical composition of the experimental rations are presented in Table 1. Complete rations and fresh water were offered twice daily at 8 am and 5 pm in quantities sufficient to allow free choice access to the ration. The growth phase lasted 120 days, the animals were weighed monthly to calculate total and daily gain and feed conversion.

Digestion and nitrogen balance trials: At the end of growth trial, four animals from each group were used to evaluate the experimental rations through digestion trials (21 days for adaptation and 7 days for sample collection). The animals were fed individually in metabolic cages. Feces samples were collected daily. Feces samples were dried at 60-70°C in a hot air oven. Volume of 50 mL diluted sulfuric acid (10%) was butted in urine collect containers each day. A representative samples (10%) of urine volume were stored for nitrogen determination. The orts were weighted daily, meanwhile feed intake was calculated. The dried samples of feces and feeds were grinded to pass through 1 mm screen and then these samples of rations and feces were stored for chemical analysis. Consequently, the nutrients digestibility and nutritive values of the experimental rations were calculated as well as nitrogen balance.

Rumen parameters: Rumen liquor samples were taken just before morning feeding, 3 and 6 h post feeding. Samples of rumen liquor were strained through 4 layers of cheesecloth and its

pH was immediately measured after collection using pH meter (HANA, Italy). Strained rumen liquor samples were acidified with 0.1 N hydrochloric acid and concentrated orthophosphoric acid and stored by freezing for determination of total volatile fatty acids (TVFA's). Ammonia was separated from rumen liquor by steam distillation, collected in boric acid solution and determined by titration with standard acid (Preston, 1995). Total VFA was determined by steam distillation according to Cunniff (1997).

Chemical analysis: Feeds and feces were analyzed for proximate chemical analyses according to AOAC (2000). Nitrogen free extract was calculated by difference. Also urine nitrogen concentration was analyzed according to AOAC (2000). The NDF, ADF and ADL was determined according to Van Soest *et al.* (1991). Cellulose and hemi-cellulose were calculated by difference according to following equation:

$$\text{Cellulose} = \text{ADF}-\text{ADL} \text{ and hemi-cellulose} = \text{NDF}-\text{ADF}$$

Carboxymethyle cellulase (CMCase) activity assay was determined in culture medium of *T. reesei* and *T. viride* according to (Mandels and Waber, 1969).

Blood parameters: At the end of growth trial, blood samples were taken from all experimental animals. A sample of 10 mL of blood per animal was withdrawn from the jugular vein. The blood sample was directly collected into a clean dried glass culture tubes (after addition of heparin as an anti coagulant) at 3 h post feeding. The blood plasma was obtained by centrifuging the blood samples soon after collection at 4000 rpm for 15 min. Blood plasma was transferred into a clean dried glass vials and then stored in deep freezer at -20°C for subsequent specific chemical analysis. Blood plasma samples were analyzed using commercial kits. Total plasma protein concentrations was determined as described by Armstrong and Carr (1964), albumin concentrations was determined using methods of Doumas *et al.* (1971) blood plasma urea was determined according to (Patton and Crouch, 1977) blood plasma cholesterol was determined according to (Raltiff and Hall, 1973). Alanin amino transferase (ALT) and aspartate amino transferase (AST) activities were calorimetrically determined according to AST and ALT kits (Quimica Clinica Aplicada S.A., Spain) based on reaction of Young (1997) Globulin was calculated.

Statistical analysis: The obtained data were statistic analyzed according to statistical analysis system (SAS., 1999). Separation between means was carried out by using Duncun Multiple Range test (Duncan, 1955). The collected data were statistically analyzed according the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where, y_{ij} is represents observation, μ is overall mean, T_i is effect of treatment (experimental group), e_{ij} is experimental error. The result are significant at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition and recovery rate: Biodegradation of rice straw resulted in reduction of OM, NDF, ADF and cellulose content for TRSV and TRSR, respectively compared to URS (Table 1). This may be attributed to utilization of soluble and structure carbohydrate contents by

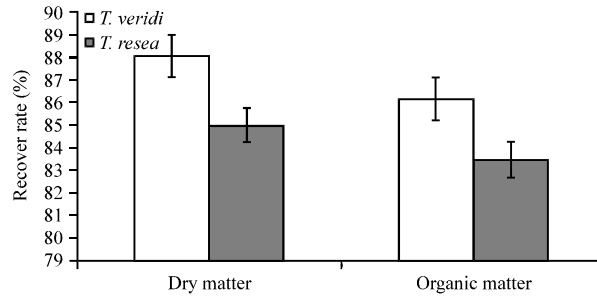


Fig. 1: Effect of biological treatment on DM and OM recovery rate

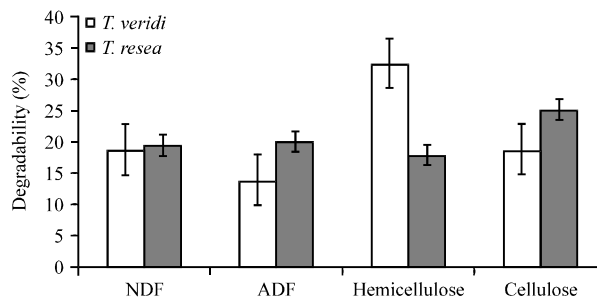


Fig. 2: Effect of biological treatment on degradability of crude fiber and its constituents

fungi cells as carbon source to produce energy and CO₂, to grow up and produce fungal biomass. In this connection Zadrazil *et al.* (1999) and Sarnklong *et al.* (2010) stated that cellulose, hemicelluloses and other solid substrates decreased after incubation of fiber materials with pure or mixed cultures of microorganisms under controlled conditions, with the aim of producing a high quality standardized products (different from composting) but losses due to mycelial growth depend on the fungus species.

On the contrary crude protein content increased by 91.7 and 28.5% for TRSV and TRSR, respectively compared to URS (Table 1). This may be due to the trapping of excess ammonia in the biological medium and its subsequent conversion to fungal protein (Gupta *et al.*, 1988) and or presents N₂-fixers bacteria in the treated rice straw which Perez *et al.* (2002) reported that most of the cellulolytic microorganisms establish synergistic relationships with non-cellulolytic species in cellulosic wastes. The present results agree with those obtained by Adamovic *et al.* (1998), Ramirez-Bribiesca *et al.* (2010) and Mansour *et al.* (2014) who found that OM, NDF and ADF contents decreased and crude protein and ash contents increased in treated crops residuals compared to the untreated.

The present results indicated that both biological treatments decreased recovered DM and OM compared to untreated rice straw. These may be due to the hydrolysis effect of the *Trichoderma reesei* and *Trichoderma viride* which fungi decompose most of solid substrate of rice straw. The same trend was observed by Jung *et al.* (1992) and Gupta *et al.* (1993), who found that, although CP content increased to 13 to 14%, the DM loss was more than 25% and was also uneconomical. Recovered DM and OM for TRSV were higher than those for TRSR (Fig. 1). Moreover *T. reesei* recorded higher capability of NDF, ADF and cellulose degradation compared to *T. viride* (Fig. 2). The lower recovery rate and higher nutrients degradation recorded for TRSR compared to TRSV

Table 2: Effect of treatments on nutrients digestibility and nitrogen efficiency utilization

Items	T1	T2	T3	SEM
Nutrient digestibility				
Dry matter	63.10	63.53	62.79	1.23
Organic matter	65.28	66.05	64.87	1.11
Crude fiber	47.55	47.68	46.83	1.17
Either extract	70.89	73.06	70.37	1.92
Crude protein	57.72	58.28	55.89	2.14
Nitrogen free extract	71.82	74.72	75.90	1.03
Rations feeding values (%)				
Digestible protein	7.21	8.16	7.26	0.29
TDN	64.90	66.91	67.30	1.05
Nitrogen efficiency utilization				
Nitrogen intake (g day ⁻¹)	15.820	17.730	16.410	-
Fecal nitrogen (g day ⁻¹)	6.780	7.435	7.335	0.6178
Digested nitrogen (g day ⁻¹)	9.040	10.295	9.075	0.6178
Urinary nitrogen (g day ⁻¹)	4.345	4.775	4.155	0.3046
Nitrogen retention (g day ⁻¹)	4.695	5.515	4.920	0.5282

The values in this table revealed the data collected from the digestion trails

(Fig. 1 and 2) may be due to that *T. reesei* had higher CM Case activity compared to *T. viride* (Table 1). Similar results were obtained by Tangnu *et al.* (1981) who found an increase of about of 2.84 and 25 times, respectively in filter paper activity and β -glucosidase activity for *Trichoderma reesei* compared to *Trichoderma viride*.

Nutrients digestibility and Nitrogen efficiency utilization: Insignificant differences were noticed among the different experimental groups in all nutrients digestibility (Table 2). Many studies indicated that the biological treatments tend to increase *in vitro* digestibility of treated materials as Zadrazil and Brunnert (1980), Nikhat *et al.* (1983), Kamra and Zadrazil (1988), Zadrazil and Puniya (1995), Sharma and Arora (2010) and *in vivo* digestibility Chen *et al.* (1995), Akinfemi *et al.* (2009) and Arora and Sharma (2009). While the insignificant effect in nutrients digestibility recorded in this study may be attributed to that neither untreated rice straw nor treated rice straw was the only roughage source in the animal ration, hence the experimental rations contained about 19% high quality roughage material (clover hay) (Table 1).

Moreover insignificant increase in feeding values as digestible protein and Total Digestible Nutrients (TDN) as well as nitrogen retention were observed for the groups received rations containing treated rice straw (TRSV and TRSR) compared to control group (Table 2). These could be understood through the higher CP content recorded for the rations contained TRSV and TRSR (Table 1) parallel to the insignificant differences in nutrients digestibility. These results disagree with the findings obtained by Walli *et al.* (1988), Fazaeli *et al.* (2004), Kabirifard *et al.* (2007), Omer *et al.* (2012), Shrivastava *et al.* (2012) and Mahesh and Mohini (2013) who found that utilization of biological treated crop residues in animal feeding resulted in a positive response in terms of nitrogen balance.

Rumen liquor parameters: Rumen liquor TVFA's, ammonia-N concentration and pH values showed insignificant differences among the different experimental groups at 0, 3 and 6 h. post feeding (Table 3). Total VFAs concentration in the rumen depends on factors such animal diets, rate

Table 3: Effect of treatments on rumen liquor parameters

Time	T1	T2	T3	Mean	SEM
TVFA's meq dL⁻¹ (h)					
0	6.88	6.60	6.04	6.51 ^c	0.470
3	13.34	12.82	12.67	12.94 ^a	0.529
6	8.88	8.96	8.24	8.69 ^b	0.459
NH₃-N values mg dL⁻¹ (h)					
0	24.33	24.85	31.19	26.79 ^c	1.440
3	34.69	34.13	36.50	35.10 ^a	1.470
6	35.67	28.87	32.54	32.36 ^b	1.470
pH values (h)					
0	7.02	6.82	6.85	6.90 ^a	0.550
3	5.80	6.02	5.75	5.86 ^c	0.550
6	6.17	6.50	6.25	6.31 ^b	0.063

^{a, b and c}Means with different superscripts in the same column are significant (p<0.05) different

Table 4: Effect of treatments on some blood plasma parameters

Items	T1	T2	T3	SEM
Total proteins (g dL ⁻¹)	7.52	7.60	7.70	0.30
Albumin (g dL ⁻¹)	3.60	3.64	3.86	0.28
Globulin (g dL ⁻¹)	3.92	3.96	3.84	0.13
Cholesterol (mg dL ⁻¹)	45.27	43.90	44.61	4.77
Urea (mg dL ⁻¹)	38.45	38.25	38.74	3.39
Creatinine (mg dL ⁻¹)	0.77	0.67	0.68	0.07
ALT (units/liter)	24.75	26.75	29.40	1.41
AST (units/liter)	71.33	65.88	63.67	2.51

^{a and b}Means with different superscripts in the same column are significant (p<0.05) different

of absorption, time of sampling, as well as the microbial population in the rumen and their activities (McDonald *et al.*, 1995). A higher pH is favorable for bacterial adherence, an important prerequisite for fiber digestion (Palmonari *et al.*, 2010). The observed insignificant differences for TVFA's, ammonia-N concentration and pH value among the different experimental groups may be attributed to that the three experimental rations were approximately similar in chemical composition (Table 1) as well as the insignificant differences recorded in nutrient digestibility (Table 2). These results agree with the findings obtained by Khattab *et al.* (2009) who reported insignificant differences in rumen fluid TVFA's concentration between animals received ration containing biological treated wheat straw and those received untreated wheat straw. However, Gado *et al.* (2006), Salman *et al.* (2008), Akinfemi and Ladipo (2011), Omer *et al.* (2012) and Mahesh and Mohini (2013) reported that biological treatments increased rumen TVFA's and ammonia concentration compared to the untreated.

Blood parameters: Data of Table 4 showed insignificant (p>0.05) increase in total plasma protein and albumin concentration for the groups received treated rice straw compared to control. These may be due to the higher CP and DCP content of the ration of these groups compared to control group. Serum total proteins reflect the nutritional status of the animal and it has a positive correlation with dietary protein (Kumar *et al.*, 1980). Also, Bush (1991) reported a positive correlation between dietary protein and plasma protein concentration and stated that the low level of plasma proteins may be attributed to a decrease in the protein absorbed and synthesized and an

Table 5: Effect of treatments on lambs growth performance and economic efficiency

Items	T1	T2	T3	SEM
Body weight (kg)				
Initial weight	23.80	22.50	23.73	1.86
Final weight	38.40	40.00	41.67	1.98
Total weight gain (kg)	14.60 ^b	17.50 ^a	17.93 ^a	0.74
Average daily gain (kg)	0.122 ^b	0.146 ^a	0.149 ^a	0.006
Feed intake				
Dry matter intake (kg h ⁻¹ day ⁻¹)	1.087	1.084	1.085	
Crude protein intake (g h ⁻¹ day ⁻¹)	137.5	150.0	141.3	
Total digestible nutrients intake (g h ⁻¹ day ⁻¹)	705	726	730	
Feed conversion (kg/kg gain)				
Dry matter conversion (kg/kg gain)	9.03 ^a	7.46 ^b	7.31 ^b	0.43
Crude protein conversion (g kg ⁻¹ gain)	1143 ^a	1031 ^{ab}	951 ^b	56
Total digestible nutrients conversion (kg/kg gain)	5.862	4.990	4.917	0.28

^a ^{ab} ^b Means with different superscripts in the same row are significantly (p<0.05) different

increase in protein losses. The present results agree with those obtained by Khattab *et al.* (2009) who found that inclusion of treated wheat straw in lamb ration did not affect total blood protein concentration. Also insignificant differences were observed among the different experimental groups for blood plasma urea and creatinine concentration. These results indicated that the experimental animals were not in a catabolism situation and kidney function was not adversely or negatively affected by biological treatments consequently, the animals were in a good nutritional condition.

Circulating cholesterol level is known to be influenced by thyroid function (Laird, 1972) and the activity of steroid producing organ (Roussel *et al.*, 1982), therefore, the non-significant variations in cholesterol level among the different treatments indicated that the activity of thyroid and steroids production was the same for all experimental treatments.

The present values of AST and ALT activity indicated normal activity of the animal hepatic tissues. Consequently, biological treatments applied in the present investigation had no an adverse effect on the liver function. Which Benjamin (1984) reported that the enzymes of AST and ALT are most important indicator for liver activity.

Growth performance: Total gain and average daily gain increased (p≤0.05) at a rate of about 22.8 and 19.9% for groups received TRSV and TRSR, respectively compared to control group. Also significantly (p≤0.05) improvements in feed conversion as DM and CP were observed for the groups received ration containing treated rice straw (T2 and T3) compared to the control group (T1), while insignificant differences were noticed in feed conversion as TDN (Table 5). The higher total gain and average daily gain recorded for groups received biological treated rice straw could be attributed to the insignificant higher intake of CP and TDN for groups T2 and T3 compared to T1. Moreover the superiority of feed conversion as DM and CP for T2 and T3 could be attributed to the close values of feed intake for the different experimental groups parallel to the higher values of average daily gain recorded for T2 and T3 compared to T1. Present findings are in consistent with the findings of Walli *et al.* (1988), Kabirifard *et al.* (2007), Khattab *et al.* (2009), Omer *et al.* (2012) and Shrivastava *et al.* (2012) who reported a positive response in terms of nutrient utilization, nitrogen balance as well as gain in body weight associated with utilization of biological treated crop residues in animal feeding.

CONCLUSION

Biodegradation of rice straw using *T. viride* and *T. reesei* improved its chemical composition which decrease fiber contents and increase its protein contents as well as no adverse effects were noticed on nutrients digestibility, rumen fermentation and nitrogen balance. Also, biological treatment upgrade the chemical composition and feeding values of rice straw in order to increase its utilization and decrease environmental problems. But it must be taken in consideration the recovery rate and the alternative solutions.

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