IMPROVING THE NUTRITIVE VALUE OF RICE STRAW BY FUNGAL TREATMENTS FOR FEEDING SHEEP.

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SUMMARY

The objective of the present investigation was to study the effect of four fungal treatments on nutritive value of rice straw and its effect on performance of Ossimi lambs. Twenty-four Ossimi lambs, 4-5 months old, having an average live body weight of 18.1±0.2 kg/h were divided according to live body weight into four groups R1, R2, R3 and R4 (six animals each). The groups were assigned at random to receive one of the four experimental rations. Animals of group R1 were fed mixture of untreated rice straw + concentrate feed mixture (CFM) as a total mixed ration (TMR) and served as control. The animals of group R2 were fed rice straw treated with *Trichoderma reesei (T. reesei)* + CFM. Animals in group R3 were fed rice straw treated with *Penicillium funiculusms (P. funiculusms)* + CFM. Whereas, animals in group R4 were offered rice straw treated with *T. reesei* and *P. funiculusms* + CFM. All fungal treatments in the present study decreased (P<0.05) NDF, ADF and ADL however, increased CP, ash, cellulose and hemicelluloses contents. At the end of the growth trial, digestibility trail were conducted to determine the digestibility coefficient and nutritive value of the tested rations and their effects on fermentation in the rumen and some blood metabolites using three animals from each group. The results revealed that the fungal treatments recorded highest digestibility coefficients (P<0.05) for all nutrients and nutritive value of R4 were higher (P<0.05) compared with control. Nutrients digestibility coefficients, fiber fractions digestibility and nutritive value compared with R2 and R3. Also, the concentrations of ruminal ammonia nitrogen, total volatile fatty acids (TVFA's) and serum urea concentration were significantly higher (P<0.05) for all fungal treatments than those of control (R1). The fungal treatments recorded better feed conversion (g DM/g gain) for R4, R2 and R3, respectively compared with the control (R1).

Keywords: *fungal treatments; rice straw; digestibility coefficients; feeding value; Ossimi lambs; performance.*

INTRODUCTION

Cereal crops generate large amount of organic agricultural waste in many countries. Cereal straws have an economical value and their residues are utilized mainly in cattle production as feedstuff and/or as bedding (Adamovic *et al.*, 1998). In Egypt, there are about 30 million tons of agricultural residues available per year. Rice straw is a major crop residue in surplus amounts (Al-Asfour, 2009).

Residues are burned or wasted, and hence lead to environmental pollution and health hazards. The main factors limiting the utilization of crop residues are their low digestibility, low protein content and some time low palatability. Rice straw has always been used as ruminant feed, since its quantities increase every year. However, it has several nutritional limitations for ruminants, because it has a low crude protein (CP) content, high crude fiber (CF) and low digestibility coefficients. Several experiments have been carried out on non-protein nitrogen treatments to increase its protein content (Langer and Bakshim, 1987).

Locally produced feeds are not sufficient to meet the nutritional requirements of livestock in Egypt (Abou-Akkada, 1988). Encouraging results obtained from using by-products in animal diets could help in reducing the shortage of animal feeds and subsequently increase milk and meat production. However, the nutritive value of the agricultural by-products like rice straw can be enhanced through their biological treatment and hence they can play an important role to meet nutrient requirements of the animals and to avoid pollution resulting from chemical treatments.

This study aimed to investigate, the ability of fungal treatments to improve nutritive value as total digestible nutrient (TDN) and digestible crude protein (DCP) of rice straw as a crop residue and its effect

on chemical composition, nutrient digestibility, some rumen and blood parameters of sheep fed fungal treated rice straw.

MATERIALS AND METHODS

Microorganisms:

Trichoderma reesei and *Penicillium funiculusms* were obtained from the Microbial Chemistry Department, National Research Center, Dokki, Cairo, Egypt. The microorganisms were maintained on YMP agar medium. The inoculum were used to inoculate 500 ml capacity conical flasks containing 20g of cooled sterilized residue by (autoclaving at 121°C for 30 minutes) moistened by basal medium containing 4% molasses, 4% urea, 0.2% KH_2PO_4 and 0.03 $MgSO_4.7H_2O$ and additives 0.5 kg yeast/ton in solid liquid ratio 1:2 by 10% (v/w) (El-Ashry *et al.*, 2002). The inoculated flasks were incubated in controlled temperature incubator at 30 °C±2 in rotary shaker 150 rpm for 48 hrs. These inoculums were used to inoculate 50 liters fermentor containing 40 liters of sterilized medium containing the composition of the same above mentioned medium by 10% (v/v) then incubated for 72 hrs. to produce 480 gm fungal biomass.

Crop residues:

Rice straw was chopped into 3-5 cm. The rice straw was strained until the moisture level reached 65-70% then treated by biological treatments layer by layer in order and left 30 days in a moderate temperature (28-30 °C).

Growth trial:

Twenty-four growing male Ossimi lambs were distributed into four similar groups (6 lambs in each), according to their weight. Average initial live body weight was 18.1±0.2 kg/head (4-5 months old). The groups were fed at random the four respective rations in 2 meals/day (8 a.m. and 3 p.m.) in groups as follows: R1: CFM + untreated rice straw (control), R2: CFM + treated rice straw with *T. reesei*, R3: CFM + treated rice straw with *Penicillium funiculusms* and R4: CFM + treated rice straw with *T. reesei* + *P. funiculusms* for 180 days. All lambs were given mixed from CFM and untreated or fungal treated rice straw [50% CFM: 50% rice straw, as TMR] to cover their growth requirements according to NRC (1985). The trial lasted for six months during which body weight and feed intake were recorded.

Digestibility trial:

At the end of feeding trial, three animals from each group were used to carry out four digestibility trials. Preliminary period lasted 21 days followed by 7 days for collection period. Feed intake and feces voided were determined to calculate digestibility coefficients. The experimental animals were fed the experimental rations (as TMR) to cover their growth requirements (NRC, 1985). Feces were collected quantitatively every day during the collection period. At the end of the collection, feces samples of each ram were ground mixed well and kept in the refrigerator for chemical analysis. Samples of feed and feces were analyzed according to A.O.A.C. (1990). Rumen liquor samples were taken from each animal at the end of collection period at 4 hours after feeding by a rubber stomach tube. Rumen liquor pH was immediately determined by pH meter. Also, ammonia nitrogen (NH_3-N) was immediately determined by the micro-diffusion method of Conway (1963). Frozen rumen liquor samples were analyzed for total volatile fatty acids (TVFA's) by steam distillation according to Abou-Akkada and Osman (1967).

Blood samples were taken from each animal at the end of collection period before feeding from the jugular vein in a clean tubes .Blood serum was separated by centrifugation and stored frozen until chemical analysis Serum total protein was determined according to Armstrong and Carr (1964); albumin according to Doumas *et al.*, (1971); GOT and GPT according to Reitman and Frankel (1957) and urea according to Siest *et al.*, (1981).

Chemical composition of feeds, residual feed and feces were determined according to A.O.A.C (1990) method. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the methods of Van Soest (1982).

The data were statistically analyzed according to Sendecor and Cochran (1980) using SAS (1985) programme. The difference between means was tested by Dancan's multiple range test (1955). The model used was as follows: $Y_{ij} + \mu + T_i + e_{ij}$ Where: Y_{ij} = the observation on the i^{th} treatment

μ = Overall mean T_i = Effect of the i^{th} treatment e_{ij} = Random experimental error

RESULTS AND DISCUSSION

Chemical composition:

Chemical composition of experimental rice straw and CFM are presented in Table (1). The results showed that, the dry matter (DM), organic matter (OM), crude fiber (CF) and NFE contents of the rice straw treated with fungal substances were lower than untreated rice straw. While CP and ash contents were higher than the control. The improvement of CP content could be attributed to fungus growth (El-Ashry *et al.* 2002). Dahanda *et al.* (1994) found that the crude protein content of spent straw increased from 3.42% to 6.19%. Obviously the increase of crude protein in the white rot fungi treated straw was due to the capture of excess nitrogen by aerobic microbes and conversion of the same into biological protein during solid-state fermentation. Generally, all fungal treatments decreased the crude fiber content than that in control. Microorganisms use crude fiber as carbon sources to grow up and convert it into microbial protein.

All fungal treatments in the present study decreased NDF, ADF and ADL however, increased cellulose and hemicelluloses (Table, 1). Chawla and Kundu (1985) reported that all fungal strains significantly degraded NDF and lignin when they treated wheat straw with *Alternaria tenius*. These results are in agreement with that of Bilal (2008).

Biological treatment of rice straw reduced NDF, ADF and ADL compared with the untreated rice straw. These results might be used due to the breakdown of lignocellulose bonds where the cellulose can be hydrolyzed by fungi (El-Ashry *et al.*, 2002).

Baraghit *et al.* (2009) found increased CP and decreased CF in basal diet of sugarcane bagasse treated with many biological treatments when fed to sheep. El-Ashry *et al.* (2002) noticed that *T. viride* and *Saccharomyces cerevisiae* increased the CP and decreased the CF and fiber fractions of low quality roughages. Also, Chawla and Kundu (1985) showed that treating wheat straw with some strains of fungi supplemented with urea and ammonium sulfate mixture reduced the content of NDF, ADF and ADL with a significant increase in crude protein.

Table (1): Chemical composition (% on DM basis) of untreated and treated rice straw and concentrate feed mixture.

Item	Control	*T. reesei*	*P. funiculusms*	*T. reesei* + *P. funiculusms*	CFM
DM	90.03	87.51	88.89	87.02	88.80
OM	84.15	82.01	83.60	79.60	88.25
CP	3.01	7.44	6.65	10.55	14.30
CF	39.33	32.00	34.20	30.20	14.11
EE	1.00	1.55	1.62	1.39	2.98
NFE	40.81	41.02	41.13	37.46	56.86
Ash	15.85	17.99	16.40	20.40	11.75
NDF	59.09	55.29	57.50	52.20	30.75
ADF	50.28	45.21	48.54	41.13	10.46
ADL	25.94	17.32	21.02	13.01	4.48
Cellulose	24.34	27.89	27.52	28.12	5.98
Hemicellulose	8.81	10.08	08.96	11.07	20.29

Digestibility coefficients and nutritive value:

Nutrient digestibility coefficients and nutritive value were affected by biological treatments as presented in Table (2). All fungal treatments (R2, R3 and R4) increased the values of nutrients digestibility coefficients than those of control (R1). Ration containing combined *T. reesei* and *P. funiculusms* treated rice straw (R3) showed the highest ($P<0.05$) digestibility coefficients for all nutrients followed by of R2 and R3 respectively. These remarkable improvements in all nutrients digestibility in rations contained fungal treated rice straw compared with the control. That could be attributed to the effect of fungal treatment by *Trichoderma* fungi in up grading and positive alteration of the chemical composition of rice straw. These positive results could be also supported by the earlier investigations in using even raw rice straw in small or large ruminant's rations, which recorded positive impact in improving its digestibility coefficients of DM, OM, CP, CF, EE, NFE and the nutritive value (El-Ashry *et al.*, 2002 and Mahrous *et al.*, 2005). Baraghit *et al.* (2009) reported that biological treatments

with different fungal and bacteria strains decreased cell wall constituents of different crop residues. Also, El-Ashry *et al.*, (1997) found that, TDN content increased from 63.93 and 63.35% in untreated rice straw and corn stalk to 72.31 and 72.88% in fungal treated ones, respectively.

The fiber fractions (NDF, ADF, ADL, cellulose and hemicelluloses) digestibility coefficients and the nutritive value (TDN and DCP) of R4 were significantly higher ($P<0.05$) than the control (R1) and the other fungal treatments (R2 and R3). The results agreed with El-Ashry *et al.* (1997) and Deraz and Ismail (2001) who reported that the biological treatments had the effect of loosening lignocelluletic bonds and solublize some of the hemicellulose contents. These results were in agreement with Mahrous *et al.* (2010).

Jung and Sahlu (1985) reported that the growth rate of mixed cultures of rumen microorganisms was inhibited by free cinnamic acids and bactereriods succinogenes appears to be particularly susceptible to growth depression. The inhibitory effects of free cinnamic acids on cellulose degradation indicated selection for different microbial populations or shifts in microbial metabolism as suggested by altered proportions of VFA produced.

Table (2): Effect of biological treatments on apparent digestibility and nutritive value.

Item	Experimental rations				± SE
	R1	R2	R3	R4	
Nutrients digestibility (%):					
DM	54.15^{d}	63.47^{b}	61.80^{c}	70.42^{a}	0.27
OM	55.04^{d}	68.12^{b}	63.52^{c}	70.10^{a}	0.07
CP	58.99^{d}	66.27^{b}	64.20^{c}	69.34^{a}	0.47
CF	52.04^{c}	60.74^{b}	59.60^{c}	65.24^{a}	0.86
EE	60.81^{d}	67.71^{b}	65.62^{c}	70.52^{a}	0.56
NFE	62.21^{d}	71.48^{b}	68.60^{c}	70.22^{a}	0.40
NDF	48.08^{d}	68.11^{b}	63.98^{c}	68.11^{a}	0.18
ADF	39.04^{s}	65.49^{b}	63.02^{c}	69.24^{a}	0.20
ADL	27.42^{d}	60.12^{b}	58.05^{c}	63.19^{a}	0.58
Cellulose	47.73^{d}	64.32^{b}	62.90^{c}	67.40^{a}	0.29
Hemicellulose	55.10^{d}	68.53^{b}	64.44^{c}	71.55^{a}	0.30
Nutritive value %:					
TDN	48.10^{c}	54.98^{b}	51.58^{c}	58.90^{a}	0.70
DCP	4.53^{c}	6.69^{b}	5.55^{c}	7.94^{a}	1.03

a, b ,c and d Means with different superscripts in the same row differ significantly ($P<0.05$).

Rumen liquor parameters:

Some rumen liquor parameters are shown in Table (3). There were no significant differences among treatments for ruminal pH values. However, all fungal treatments (R2, R3 and R4) were significantly higher ($P<0.05$) in the concentrations of ruminal ammonia nitrogen and total volatile fatty acids (TVFA's) than those of control (R1). Ration containing combined *T. reesei* and *P. funiculusms* treated rice straw (R3) showed the highest ($P<0.05$) concentrations of ruminal ammonia nitrogen and TVFA's followed by of R2 and R3 respectively.

These results of fungal treatments might be, related to the more utilization of the dietary energy and positive fermentation the rumen. It is worthy to notice that the balance between NH_3-N and TVFA's concentrations reflect the pH values in the rumen liquor. These results might be related to the more utilization of the dietary energy and positive fermentation in the rumen. These results are in agreement with that of Mahrous *et al.* (2009).

Table (3): Effect of fungal treatments on some rumen liquor parameters for sheep.

Item	Experimental rations				± SE
	R1	R3	R4	R4	
pH	6.10	6.20	6.20	6.10	0.52
NH_3-N (mg/100ml)	13.28^{d}	17.62^{b}	14.80^{c}	20.68^{a}	0.12
TVFA's /meq/100ml)	8.25^{d}	11.77^{b}	10.20^{c}	15.64^{a}	0.13

a, b, c and d Means with different superscripts in the same row differ significantly ($P<0.05$).

Blood parameters:

Levels of serum urea, total protein, albumin, globulin, albumin/globulin ratio (A/G ratio), GOT and GPT for the animals fed untreated or fungal treated rice straw are presented in Table (4). Feeding animals on rations contained fungal treated rice straw (R2, R3 and R4) significantly ($P<0.05$) increased level of serum urea concentration than those fed the untreated rice straw (control or R1). This result may be supported by the finding that rumen ammonia nitrogen concentrations were higher ($P<0.05$) in the fungal treated rations as compared with control (Table, 3). The apparently normal values obtained in the present study for blood serum urea-nitrogen suggests efficient utilization of nitrogen in the different experimental rations by rumen microorganisms.

However, there were no differences among all treatments for the levels of serum total protein and its fractionations. The ranges of serum total protein and its fractionations values were within those normal ranges. Cornelius (1970) reported that the concentration of the total protein in serum of animals ranged between 6-10g/dl serum. These results of blood metabolism are in agreement with those reported by Deraz and Ismail (2001) and Mahrous *et al.* (2010).

It is important to note that all values of A/G ratio were higher than 1.0, which indicates that animals did not suffer from any health problems that might affect the performance of the experimental animals.

Several factors affect GOT and GPT enzymes; as activities as feeding practices, environment, genetic control, response to stress, age, liver function and body weight (Boots *et al.*, 1969). It is clear that the experimental treatments did not significantly affect serum GOT and GPT levels in the experimental sheep. In general, the values recorded for GOT and GPT were within the normal range reported by Abd El-Kareem (1990) who found that values of GOT and GPT ranged from 24 to 65 and from 19 to 37 U/L, respectively in goats.

Table (4): Effect of fungal treatments on some blood parameters for sheep.

Item	Experimental rations				± SE
	R1	R2	R3	R4	
Urea (mg/100ml)	21.20^{d}	24.85^{b}	22.85^{c}	26.45^{a}	0.20
Total protein (gm/dl)	7.32	7.40	7.35	7.45	0.12
Albumin (gm/dl)	3.90	3.84	3.78	3.80	1.22
Globulin (gm/dl)	3.60	3.56	3.57	3.65	1.20
A/G ratio	1.08	1.07	1.05	1.04	0.38
GOT (U/L)	30.60	30.70	30.51	30.28	0.09
GPT (U/L)	22.46	22.50	22.35	22.16	0.91

a, b ,c and d Means with different superscripts in the same row differ significantly ($P<0.05$).

Growth performance:

The average DM intake expressed as (g/h/d), average daily body gain (ADG) and feed conversion of the experimental groups are presented in Table (5). The results revealed that the average DMI as (g/h/d) of lambs during 180 days of the experimental period was higher for lambs, fed *T. reesei* + *P. funiculusms*, treatment (1258 g/h/d) followed by *T. reesei* treatment (1210 g/h/d) and treated *P. funiculusms* (1170 g/h/d) than the control (1100 g/h/d). The results of feed conversion (g DM/g gain) showed that the combination between fungus recorded the best value (7.51) followed by the *T. reesei* treatment (7.71) and *P. funiculusms* (8.44) than the control (8.84).

Table (5): Effect of fungal treatments on feed intake and feed conversion of experimental animals.

Item	Experimental ration			
	R1	R2	R3	R4
No. of animals	6	6	6	6
Experimental period (days)	180	180	180	180
Initial weight (kg)	18.10	18.20	18.10	18.20
Final weight (kg)	40.50	45.50	43.90	48.32
Total gain (kg)	22.40	27.30	25.80	30.12
Average daily gain [ADG] (g)	124.4	151.6	143.3	167.3
DMI (g/d)	1100	1170	1210	1258
Feed conversion (g DM/g gain)	8.84	7.71	8.44	7.51

These results agree with those obtained by Bassuny *et al.* (2003) who found that significant differences in feed units intake may be due to the differences in the nutritive values and dry matter intake between the tested groups. Feed conversion in all biologically treated rice straw were the lower than the control. Mohamed *et al.* (1998) indicated that the feed conversion of lambs fed rice straw treated with fungus was better compared with untreated rice straw.

The overall results obtained in this study revealed that the fungal treatments of rice straw by *T. reesei + P. funiculusms, T. reesei* and *P. funiculusms* increased protein content, protein digestibility, fiber fractions digestibility. The recycling of agricultural wastes is important to raise its nutritional value and can be used in the ruminants feeding. Biological treatments can utilize lignin along with cellulose and other components of the substrate; these organisms grow slowly and degrade the structural carbohydrates of crop residues. In addition, biological treatments as a result of molecular biology are preferable in terms of being a biological treatment, rather than the other treatments such as chemical and physical treatments for better and clear environment.

CONCLUSION

Mixture of *Trichoderma reesei* and *Penicillium funiculusms* could be successfully used to enrich rice straw with protein and improve nutrients digestibility and nutritive value of rations containing fungal treated rice straw without any adverse effects on animal performance and health.

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تحسين القيمة الغذائية لقش الأرز بواسطة المعاملات الفطرية و استخدامه فى تغذية الأغنام.

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تهدف هذه التجربة الى دراسة مدى تأثير المعاملة بالفطريات على تحسين القيمة الغذائية لقش الأرز و مردود ذلك على أداء الحملان الأوسيمى. و لذلك تم استخدام عدد 24 من الحملان الأوسيمى عمر 4-5 شهور بمتوسط وزن 18.1 ±0.2كيلوجرام قسمت وفقا لوزن الجسم إلى أربعة مجموعات تجريبية (ستة حملان فى كل مجموعة). وزعت المجاميع الأربع عشوائيا للتغذية على إحدى العلائق التجريبيه الأربع حيث تغذت حيوانات المجموعة الأولى على مخلوط من قش الأرز الغير معامل و العلف المركز، المجموعة الثانية على مخلوط من قش الأرز المعامل بفطر *Trichoderma reesei* و العلف المركز، المجموعة الثالثة على مخلوط من قش الأرز المعامل بفطر *Penicillium funiculusms* و العلف المركز، و المجموعة الرابعة على مخلوط من قش الأرز المعامل بـ (*Penicillium Trichoderma reesei+funiculusms*) و العلف المركز أدت جميع المعاملات الفطريه إلى الإقلال من تركيزات ADL , ADF , NDF بينما رفعت تركيزات البروتين والرماد والسليولوز والهيميسليولوز .تم أجراء تجارب الهضم فى نهاية التجربة و ذلك باستخدام 3 حيوانات من كل مجموعة لتقدير معاملات الهضم و القيمة الغذائية للعلائق المختبرة مع دراسة بعض خصائص التخمر فى الكرش وبعض دلائل التمثيل الغذائى فى الدم.

و أوضحت النتائج ان المعاملات الفطرية كانت ذات معاملات هضم اعلي معنويا (عند مستوى 5%) لمعظم العناصر الغذائيه والقيمة الغذائية مقارنة بمجموعة الكنترول (المجموعة الأولى). و في ذات الوقت كانت المجموعة الرابعة متفوقة على المجموعة الثانية تليها المجموعة الثالثة فى معاملات الهضم للمواد الغذائية وايضا لمكونات الالياف والقيمة الغذائية. كما سجلت المعاملات الفطرية ارتفاع فى قيم تركيز الامونيا و الاحماض الدهنية الطيارة و ايضا تركيز اليوريا فى سيرم الدم و أيضا سجل نفس الاتجاه لمعاملات التحويل الغذائى حيث كانت قيم المعاملات الفطرية الرابعة ثم الثانية ثم الثالثة على الترتيب أفضل القيم مقارنة بالمجموعة المقارنة (الأولى).