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# EFFECT OF REPLACING CLOVER HAY WITH GRADED LEVELS OF BIOLOGICALLY-TREATED RICE STRAW ON NUTRITIVE VALUE AND RUMEN FERMENTATION IN SHEEP

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**ABSTRACT:** *This study was conducted to evaluate the effect of inclusion of fungal-treated rice straw in sheep ration as a replacement for some of Berseem-hay on digestibility, nutritive value, N- balance and rumen fermentation. Twelve Barki rams were assigned randomly to three similar groups (4 rams each) with an average body weight of 45 kg. Treated rice straw was included in three rations with 50:50% roughage: concentrate ratio at different replacement percentage of clover hay (0, 20 and 40%). Rumen fermentation characters (pH, VFA and $NH_3$-N) were determined at 0, 3 and 6h after feeding. Digestion coefficients of CP, CF and EE were increased with the treated rations. The experimental diet with 40% RS recorded the highest values of both DCP% and TDN%. ADF and CEL digestibility were significantly higher ($P<0.05$) for RS40 than the other diets. Nitrogen balance (NB) was 4.3, 4.94 and 6.04 g/d, for control, RS20 and RS40, respectively. Differences were highly significant ($P<0.001$). Treating rice straw did not negatively affect the rumen fermentation.*

***Key words*:** *Rice straw, biological treatment, nutritive value, fiber fraction, rumen fermentation.*

## INTRODUCTION

In Egypt, there is a great shortage of feedstuffs particularly during summer season and early autumn (Yousef, 2005). The amount of the agricultural crop residues in Egypt ranging from 30 -35 million tons a year of which only 7 million tons as animal feed and 4 million as organic manure are being utilized (Abou Hussein and Sawan, 2010). The most abundant roughages in Egypt are rice straw, wheat straw, bean straw, corn stalks and stover, corn cobs, rice hulls and sugarcane bagasse. These in turn are being burned and used as a raw material for paper or fuel, composted, or in small percentages used for feeding animals (Kuhad *et al*., 2013). The main factors limiting the utilization of crop residues as animal feed are their low digestibility, low protein content and sometime low palatability (Abd El-Rahman *et al*., 2014). Rice straw is high in cellulose, hemicellulose, and lignin, but low in pectin and silica. An important limiting factor in using by-products for animal feeding is their low digestibility due mainly to non-polysaccharide components such as phenolic acids (Ortega *et al*., 1986; Kuhad *et al*., 2013; Elghandour *et al*., 2014). Utilization of such by-products can not only be used in favor of solving feed shortage problem but also as a method to control environmental pollution (Zaza, 2004). Biological methods, including the use of white rot fungi and its enzymatic extracts, have the potential to eliminate the problems associated with physicochemical methods and appear to be the most promising in delignification (Tuyen *et al*., 2012; Arce-Cervantes *et al*., 2013). White-rot fungi are capable of decomposing and mineralizing plant cell components because, during fungal colonization of a suitable substrate, the easily digestible carbohydrates are converted into simpler sugars (Khattab *et al*., 2013; Kholif *et al*., 2014). This is known as the fungus' primary metabolism. These sugars are totally consumed by the fungus and afterward secondary metabolism is initiated, which consists of the breakdown of structural carbohydrates and lignin from substrates by extra-cellular enzymes like laccase, manganese peroxidase and lignin peroxidase (Kuhad *et al*., 2013). Application of rice straw for plantation of mushrooms is well known in Egypt. In this respect the consumption of mushroom in Egypt is quite low; therefore, the amount of straw used by this way remains very limited but still feasible and promising. The objective of this study was to evaluate the effect of inclusion of fungal-treated rice straw in sheep ration as a replacement for some of Berseem-hay on digestibility, nutritive value, N- balance an rumen fermentation.

## MATERIAL AND METHODS

The present study was carried out at Department of Animal Production, Faculty of Agricultural, Menoufia University in order to study the effect of treating rice straw with edible mushroom to improve its nutritive value as feed resources for ruminants and possibility of replacing clover hay.

Rice straw was immersed into boiling water; inoculated with fungus and incubated for 2 weeks. Twelve Barki rams were assigned randomly to three similar groups (4 rams each) with an average body weight of 45 kg. The experimental design used in this study was complete randomized block. The first two weeks were considered as an adaptation period, the consecutive 10 days were used for samples collection. The experimental animals were kept individually in metabolic crates (1.60m x 0.53m) as described by Maynard *et al*. (1979) allowing separate collection of urine and feces. Treated rice straw (TRS) were included in three rations with 50:50% roughage: concentrate ratio at different replacement percentage of clover hay (0, 20 and 40%): i) Control; containing 629 g clover hay + 622 g concentrates; ii) RS20; containing 503.2 g clover hay+ 125.8 g TRS + 622g concentrates; iii) RS40 containing 377.4 g clover hay + 251.2 g TRS +622 g concentrates. Rations were calculated according to NRC (1985) requirements. These rations were formulated to be equal in nitrogen content by controlling formulation of concentrate diet (corn, wheat bran, cotton seed meal and soybean meal). The experimental rations were offered twice daily at 09:00 and 16:00 h. All animals had free access to fresh water.

Feces were quantitatively collected at 9:00 a.m. before feeding. A quantity of 10% from feces was withdrawn and dried to a constant weight in a forced air oven at 70ºC for 24 hrs. Dry fecal samples were ground to pass a 2mm screen and kept in plastic bags for later analysis. Digestibility of all nutrients was determined.

For the determination of nitrogen balance, every day during the collection periods, urine was quantitatively collected at 9:00 a.m. before feeding. Urine was collected in containers containing 50 ml of 0.1N HCl to maintain *p*H<2.00 in order to avoid N loss through ammonia volatilization and to avoid bacterial growth in the urine. A quantity of 10% of the total urine from each ram was withdrawn and kept in glass bottles in the freezer for later determination of N content.

In order to determine the rumen fermentation characters (*p*H, VFA and $NH_3$-N), samples of rumen fluid were obtained at (0, 3 and 6) after feeding. The rumen samples were collected using rubber stomach tube inserted into the rumen via the esophagus. Rumen *p*H was measured immediately after collection using a digital *p*H meter (Sophisticated microprocessor *p*H meter). Rumen contents were strained through four layer of cheesecloth, and the fluid portion was acidified with 7.2 N $H_2SO_4$ at the rate of 1 ml of acid/100 ml of strained rumen fluid. Strained samples were stored frozen (-10ºC) until analyzed for ammonia-N and total volatile fatty acids. Total volatile fatty acids in the rumen liquor (VFA) were measured according to stem distillation procedure as described by Warner (1964) calculations of total volatile fatty acids. Ammonia-N ($NH_3$-N) concentration was determined according to Horn *et al*. (1981).

Samples of the untreated, treated roughage and feces were ground and subjected in duplicate to proximate analysis. Samples of daily output of feces were taken after drying at 60 °C for 24h and then were also ground. All the ground samples were stored in stoppered bottles. Dry matter (DM), crude protein (CP), and crude fiber (CF) and ash of the feedstuffs and feces samples were determined according to AOAC (1990). The nitrogen free extract (NFE) was calculated by difference. Representative samples of the experimental ration were analyzed for fiber fraction according to Van Soest and Breston (1979) to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Cellulose and hemi-cellulose were determined by difference.

The data obtained in this study were statically analyzed by analysis of variance using SPSS program version 19.

## RESULTS AND DISCUSSION

The effect of fungal treated rice straw fed to sheep on nutrient digestibility is presented in Table (1). Digestion coefficients of the experimental diets showed that the differences between the experimental diets were significant for CP, CF and EE. Digestibility of CP was 69.4, 71.85 and 74.12% for control, RS20 and RS40, respectively. The respective values for EE and CF were 72.97 and 53.17% for control, 73.82 and 54.26% for RS20 and 78.59 and 63.65% for RS40.

**Table 1: Effect of dietary treatments on nutrient digestibility (%)**

| Nutrients | Treatments | | | SEM | Sig |
|---|---|---|---|---|---|
| | Control | RS20 | RS40 | | |
| ***Digestibility, %*** | | | | | |
| DM | 69.13 | 66.20 | 68.99 | 0.72 | NS |
| OM | 71.33 | 69.3 | 72.34 | 0.67 | NS |
| CP | $69.4^{a}$ | $71.85^{ab}$ | $74.12^{b}$ | 0.85 | 0.01 |
| EE | $72.97^{a}$ | $73.82^{a}$ | $78.59^{b}$ | 0.98 | 0.01 |
| NFE | 76.07 | 72.35 | 75.66 | 0.83 | NS |
| CF | $53.17^{a}$ | $54.26^{a}$ | $63.65^{b}$ | 1.37 | 0.001 |
| NDF | 50.76 | 52.54 | 53.21 | 1.15 | NS |
| ADF | $51.62^{a}$ | $51.44^{a}$ | $56.92^{b}$ | 1.37 | 0.05 |
| CEL | $48.27^{a}$ | $49.56^{a}$ | $51.94^{b}$ | 1.11 | 0.05 |
| HEM | 60.51 | 59.21 | 60.22 | 1.18 | NS |
| ***Nutritive value*** | | | | | |
| TDN | $64.25^{a}$ | $62.86^{a}$ | $66.38^{b}$ | 0.66 | 0.05 |
| DCP | $8.28^{a}$ | $8.48^{a}$ | $8.80^{b}$ | 0.099 | 0.05 |

SEM, standard error of means
TDN, total digestible nutrients, DCP, digestible crude protein.
$^{a,b}$ means within each row with different superscript differ significantly

Digestibility of CP, CF and EE were the highest for RS40, while control diet recorded the lowest values. Differences regarding digestibility of DM, OM and NFE were not significant between the experimental groups. Digestion coefficients for DM were 69.13, 66.20 and 68.99 for control, RS20 and RS40, respectively. Those of OM were 71.33, 69.3 and 72.34% in the same respective order. Values of NFE digestibility were 76.07, 72.35and 75.66% for control, RS20 and RS40, respectively.

Higher IVDMD have been reported due to fungal treatment (Karunanandaa *et al*., 1992; 1995; Karunanadaa and Varga, 1996a,b; Fazaeli and Masoodi, 2006). Also Khattab *et al.* (2013) reported that SRS had higher *in vitro* dry matter disappearance (IVDMD) and *in vitro* organic matter disappearance (IVOMD) compared to RS. However, some researchers reported that fungal delignification causes low digestibility values (Hader *et al*., 1993; Jalc *et al*., 1996). This difference in reported results may be possibly related to many factors such as silica content of RS and fungus growth stage influence cell wall degradation and their digestibility (Jafari *et al*., 2007). Khattab *et al*. (2013) investigated the possibility of replacing berseem clover (BC; *Trifolium alexandrinum*) with SRS at different levels and indicated that the levels of 50 and 90% replacement had the highest values of DMD and OMD compared to the other levels (0, 10, 20, 30, 40, 60, 70, 80, 100%).

Fungal delignification increases the surface area of lingo-cellulosics, thus providing better opportunity to the rumen micro-organisms which not only enhances the digestibility of the feed but also improves their nutritional value (Sharma and Arora, 2013).

The differences between the experimental diets were significant (P<0.05) in nutritive values when expressed as TDN% and DCP% (Table 1). The experimental diet with 40% RS recorded the highest values of both DCP% and TDN%. The respective values for DCP% and TDN% were 8.28% and 64.25% for control, 8.48% and 62.86% for RS20 and 8.8% and 66.38% for RS40. The improvement in the nutritive value was mainly due to the increase in digestibility of CP, CF and EE. Since, Most of the protein of Berseem hay is non-soluble protein which may escapes fermentation in the rumen (Tag El-Din *et al*., 2009).

Total digestible nutrients and DCP for rice straw treated with fungus were higher than untreated rice straw (El-Ashry *et al.,* 2002 and Gado *et al*., 2006). The delignification plays an important role in improvement of the digestibility and feeding value of straw (Sharma and Arora, 2013). Khattab *et al*. (2013) concluded that treatment of RS with *Pleurotus ostreatus* improved the potential feeding value of the resultant substrates (i.e., SRS) as feed resources for ruminants and possibility of replacing BC (Berseem clover) with SRS at high levels of up to 50 or 90% from diets.

The results in Table (1) show the digestion coefficients of cell wall constituents of the experimental diets. ADF and CEL digestibility were significantly higher (P<0.05) for RS40 than the other diets. The digestion coefficients of ADF were 51.62%, 51.44% and 56.92% for control, 20% and 40% RS, respectively. Values of CEL were 48.27%, 49.56% and 51.94% in the same respective order. These results strengthen the viewpoint reported by Cohen *et al*. (2002) who indicated that during selective lignin degradation, the cellulose is exposed and can be utilized by ruminants.

Concerning NDF and HEM digestibility, differences were not significant among groups. These results differ than that reported by Rodrigues *et al.* (2008) who studied the effect of enzyme extracts isolated from white-rot fungi on chemical composition and *in vitro* digestibility of wheat straw and found that using these enzymes increased the *in vitro* NDF degradability (IVNDFD). Calzada *et al*. (1987) found that the *in vivo* digestibility of NDF and ADF were higher in straw by white rot fungi comparing with untreated wheat straw. (El-Ashry *et al*., 2003) noticed that *Trichoderma viride*, *Penicillium funiculosium* and *S. cerevisiae* significantly increased the digestibility of OM, CF, NFE, ADF, hemicellulose and cellulose of low quality roughages (banana waste, corn stalk, rice straw and wheat straw). Non-significant differences of NDF and HEM in this study may have been due to the short time of incubation of rice straw with the fungus.

Data on nitrogen balance as affected by the replacement with fungal treated rice straw are shown in Table (2). Data indicated that the nitrogen intake (NI) was almost equal being 21, 20.77 and 20.88 g/d for animals fed control diet, 20 or 40% RS, respectively. Nitrogen excreted in feces (FN) was lower ($P<0.05$) with sheep fed 40% RS than those fed control diet. Values were 6.43, 5.85 and 4.91g/d for control, RS20 or RS40, respectively. These data are in complete agreement with the digestibility of CP (Table 1). The respective values for nitrogen excreted in urine (UN) were 10.27, 9.98 and 9.93 g/d for the same respective groups. Differences were not significant. Nitrogen balance (NB) was 4.3, 4.94 and 6.04 g/d, for control, RS20 and RS40, respectively. Differences were highly significant ($P<0.001$). Feeding sheep diet contained 20% or 40% fungal treated rice straw improved NB by 14.88 and 40.47% over those fed control diet. Biological value of the protein was 29.5, 33.1 and 37.8% for the same respective groups; differences were significant ($P<0.05$). Improvement in BV for diets contained 20% and 40% was 12.2 and 28.14%, respectively. Sheep given fungal treated rice straw diets secreted less N in feces leading to higher CP digestibility therefore, higher nitrogen balance.

Results about NB and BV were similar to those found by El-Mahy (2013) who found that biological treatments improved the NB and BV of the dietary protein as a result of feeding biologically treated RS.

Gado (1997) evaluated the effect of enzymatic treatment on the apparent N retention and weight gain and reported improvement in goats fed silages treated with cellulase. Urea treated or urea treated and inoculated with *Penicillium funiculosum* of chopped roughages (rice straw and corn stalks) had higher N balance than the control diet (El-Ashry *et al.,* 1997).

**Table 2: Nitrogen balance (g/d) as affected by the replacement with treated rice straw**

| Items | Treatments | | | SEM | Sig |
|---|---|---|---|---|---|
| | Control | RS20 | RS40 | | |
| Nitrogen intake, NI (g/d) | 21.00 | 20.77 | 20.88 | 0.025 | NS |
| Fecal nitrogen, FN (g/d) | 6.43$^{b}$ | 5.85$^{ab}$ | 4.91$^{a}$ | 0.23 | 0.05 |
| Urinary nitrogen, UN (g/d) | 10.27 | 9.98 | 9.93 | 0.29 | NS |
| Nitrogen balance, NB (g/d) | 4.30$^{a}$ | 4.94$^{b}$ | 6.04$^{b}$ | 0.38 | 0.001 |
| Biological value, BV (%) | 29.50$^{a}$ | 33.10$^{b}$ | 37.80$^{b}$ | 1.96 | 0.05 |

SEM, standard error of means
$^{a,b}$ means within each row with different superscript differ significantly

Effect of the experimental diets on the rumen liquor *p*H of sheep at 0, 3 and 6 h after feeding are shown in Table (3). In general, the highest rumen *p*H values were recorded for all diets at zero time and decline thereafter to reach the least value at 3hr post-feeding and then it started to rise up at 6hr. The results indicated that rumen *p*H values were not significantly affected by different treatments except at 6 h after feeding. The differences were significant ($P<0.01$). The recorded *p*H values were within the reported ranges for normally functioning rumen being from 6 to7.

Young *et al*. (2010) used six fistulated steers to study the effects of supplementation of spent mushroom substrates (SMS) on rumen fermentation and found no major effects of different dietary treatments on rumen parameters such as *p*H. Abo Donia *et al*. (2005) reported that biological treated peanut hulls and sugarcane bagasse increased significantly ($P<0.01$) *p*H values compared with untreated rations. While others (Erdman, 1988; Kolver and de Veth, 2002) reported that decreasing the dietary CF, NDF or ADF level generally decreases rumen *p*H.

The results in Table (3) showed that concentration of total VFA was low before feeding and then increased in animals under all the experimental categories post-feeding. Before feeding, VFA was 7.89, 7.86 and 8.09 meq/dl in rumen of sheep fed control, RS20 and RS40 diets, respectively. At 3h post-feeding, VFA was 10.16, 10.09 and 10.08 meq/dl for control, RS20 and RS40, respectively; at 6h post-feeding, VFA was 10.24, 10.41 and 9.88meq/dl for the same respective order. Differences were not significantly affected by different treatments at all sampling time. These results were in agreement with that of Young *et al*. (2010) who studied the effects of supplementation of spent mushroom substrates on rumen fermentation and found no major effects of different dietary treatments on individual and total VFA production. Omer *et al*. (2012) used thirty-six male growing Rahmani lambs to determine the effects of inclusion biologically treated corn stalks (BTCS) treated with *Trichoderma ressi* (*T. ressi*) on growth performance and found that inclusion BTCS in sheep rations increased total volatile fatty acids (TVFA's) concentrations compared to control diet; suggesting that increasing TVFA's might be related to the more utilization of dietary energy and positive fermentation in the rumen.

**Table 3: Rumen fermentation parameters in sheep as affected by the replacement with fungal treated rice straw**

| Item | Sampling time, h | Control | RS20 | RS40 | SEM | Sig |
|---|---|---|---|---|---|---|
| ***p*H** | **0** | 6.78 | 6.82 | 6.87 | 0.049 | NS |
| | **3** | 6.17 | 6.29 | 6.21 | 0.039 | NS |
| | **6** | $6.44^{b}$ | $6.20^{a}$ | $6.15^{a}$ | 0.042 | 0.01 |
| **VFA (meq/dl)** | **0** | 7.89 | 7.86 | 8.09 | 0.26 | **NS** |
| | **3** | 10.16 | 10.09 | 10.08 | 0.21 | **NS** |
| | **6** | 10.24 | 10.41 | 9.88 | 0.12 | **NS** |
| **$NH_3$-N (mg/dl)** | **0** | 17.43 | 16.52 | 17.89 | 1.15 | **NS** |
| | **3** | 19.72 | 19.53 | 19.6 | 1.1 | **NS** |
| | **6** | 15.95 | 16.24 | 16.36 | 1.03 | **NS** |

It should be noted that TVFA's concentration in the rumen is governed by several factors such as dry matter digestibility, rate of absorption, rumen *p*H, transportation of the digesta from the rumen to the other parts of the digestive tract, and the microbial population in the rumen and their activities (Allam *et al*., 1984).

Results concerning $NH_3$-N concentration are presented in Table (3). It is obvious that the concentration of $NH_3$-N was lowest pre-feeding for all the experimental diets being 17.43, 16.52 and 17.89 mg/dl for control, RS 20 and RS 40%, respectively. Concentration of $NH_3$-N increased to reach the highest level at 3h post feeding being on the average 19.62 mg/dl for all groups; $NH_3$-N decreased thereafter to reach the lowest levels at 6h post feeding being on the average 16.18 mg/dl. Differences were non-significant regarding all treatments at all sampling times.

Concentration of $NH_3$-N in the rumen liquor required for maximal microbial protein synthesis has been reported to range from 15– 20 mg/dl (El-Sheikh, 2005). In the present study rumen ammonia concentrations were in the range of those reported to be required for maximum rumen microbial activity. Zewil (2005) found that the biologically treated rice straw ration tended to increase ammonia nitrogen concentration in rumen liquor. Abo Donia *et al.* (2005) reported that biological treated peanut hulls and sugarcane bagasse increased significantly (P<0.01) concentration of NH3-N in the rumen liquor compared with untreated rations. Mahrous *et al*. (2009) noted that feeding sheep on treated corn stalks containing diets recorded higher (P<0.05) $NH_3$-N concentration being 20.25, 18.65 and 22.38 mg/100ml for T2, T3 and T4, respectively, compared with that of control ration (15.30 mg/100ml). Yadov and Yadav (1988) noticed that increased rumen $NH_3$-N concentration might be due to the higher intake of nitrogen and higher CP digestibility.

Young *et al.* (2010) revealed no major effects of different spent mushroom rice straw on rumen parameters such as *p*H, ammonia-N, individual and total VFA production.

It could be concluded that the nutritive value of rice straw could be improved by the biological treatment with *pleurotus spp.* ; this treatment improved digestibility, nitrogen balance without negative effect on rumen fermentation. Treated rice straw could replace Egyptian clover hay up to 40%.

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# تأثير استبدال دريس البرسيم بمستويات متتالية من قش الأرز المعامل حيويا على القيمة الغذائية وتخمرات الكرش في الأغنام

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## الملخص العربى

أجريت هذه الدراسة لتقييم تأثير إدراج قش الأرز المعامل حيويا في علائق الأغنام كبديل لدريس البرسيم على الهضم والقيمة الغذائية وميزان النيتروجين وتخمرات الكرش. استخدم في هذه الدراسة اثني عشر من الكباش البرقي

قسمت عشوائيا إلى ثلاث مجموعات متماثلة (٤ كباش بكل منها) بمتوسط وزن الجسم من ٤٥ كجم. تم أدراج القش المعامل في ثلاث علائق تحتوي على العلف المالئ إلى المركز بنسبة ٥٠: ٥٠٪ وذلك بنسبة استبدال مختلفة من القش المعامل بديلا لدريس البرسيم (٠، ٢٠ و ٤٠٪). وتم تقدير معاملات الهضم للمكونات الغذائية المختلفة وكذلك تقدير تخمرات الكرش (درجة الحموضة، الأحماض الدهنية الطيارة ونيتروجين الأمونيا) عند ٠، ٣ و ٦ساعات بعد الاكل. زادت معاملات هضم البروتين الخام والالياف الخام الدهن عند التغذية على القش المعامل . النظام الغذائي التجريبي المحتوي على ٤٠٪ قش معامل سجلت أعلى قيمة غذائية في صورة مركبات غذائية مهضومة كلية أو بروتين مهضوم. وقد سجلت الألياف المستخلصة بالأحماض وكذلك السيليلوز هضما أعلى معنويا لنفس العليقة المحتوية على القش المعامل بنسبة ٤٠% عن العلائق التجريبية الأخرى. بلغ النيتروجين المحتجز ٤,٣ – ٤,٩٤ – ٦,٠٤ جم يوميا للعلائق التجريبية المقارنة والمحتوية على ٢٠% و ٤٠% من قش الأرز المعامل, على التوالي. وقد كانت الفروق عالية المعنوية . أظهرت النتائج أن معاملة قش الأرز حيويا لم تؤثر سلبا على مقاييس التخمر بالكرش.