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THE EFFECT OF PARTIAL REPLACEMENT OF CLOVER HAY WITH FUNGAL TREATED RICE STRAW ON GROWTH PERFORMANCE, DIGESTIBILITY, RUMEN FERMENTATION, BLOOD BIOCHEMICAL AND IMMUNE STATUS OF BARKI SHEEP

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ABSTRACT: Twenty seven Barki lambs, seven months old, with live body weight of 33.33±2.21kg were used to investigate the effects of partial replacement of berseem hay by fungal treated rice straw on growth performance, digestibility, rumen fermentation, some blood biochemical and immune status of Barki sheep. The animals were divided into three comparable groups. Three rations were prepared with the same roughage: concentrate ratio of 40:60% and iso-nitrogenous; C - Control (60% CFM + 40% berseem hay), TV- 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Trichoderma viride*), AS - 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Aspergillus fumigatus*). Lambs body weight was recorded at the start of the experiment and thereafter regularly at 14 days interval. After completion of the feeding trial (12 weeks), four animals per treatment were used in a metabolism trial. Obtained results showed that treated rice straw showed significant (P< 0.05) improvement in OM, CP, CF and NFE, while DM, EE and Ash did not differ significantly. The body weight non-significantly increased here-after up to week six; then increases started to reach the significant level. The final body weight was 43.45, 45.5 and 45.35kg for C, TV and AS groups respectively; total gain was 10.18, 12.07 and 12.14kg for the respective groups. Average daily gain was 121, 143.67 and 144.44g/d., CP, CF, NFE and EE digestibility were significantly (P< 0.05) increase in treated groups, while DM digestibility did not differ significantly by treatments. The content of NDF significantly (P < 0.05) declined with treated rice straw via either TV or AF by 10.37% and 7.21% respectively compared with URS. The same trend shows with Acid detergent fiber (ADF) which was 56.22% for the untreated rice straw than treated rice straw with either TV (50.17%) or AF (51.64%). All treatments had similar nutritive values, being 63.00, 63.83 and 63.08% for TDN and 10.11, 10.57and 10.39% for DCP, respectively. Values of NB were 4.03, 4.98 and 5.03g/d for the three experimental groups, respectively. The biological value of the dietary protein was higher for groups AF (35.66%) and TV (35.48%) and lowest for control group (30.00%). Treating rice straw with either TV or AF fungus did not affect the rumen pH values at any time of feeding. At three hours from feeding VFA production increased from 10.12 in C diet to 12.98 for TV and 12.35meq/dl for AF. Rumen NH3 concentration increased from 16.25 in C diet to 18.65 for TV and 18.39mg/dl for AF at three hours from feeding, similar trend was found at six hour post feeding. Generally, all values were within the normal values of blood characteristics of sheep. Serum total protein, albumin and glucose were significant (P< 0.05) increase in treated groups than control group, while treatments had no significant effect on globulin, urea, creatinine, AST and ALT. Immunological parameters (IgA, IgG and interleukin 2) were within normal ranges and did not differ significantly.

Key words: Replacement, rice straw, fungal treatment, performance, sheep.

INTRODUCTION

Due to the shortage of animal feeds in Egypt, intense research efforts must be focused on examining the potential for employing novel, nontraditional sources of agricultural byproducts as animal feed and enhancing their nutritional value. (Ali, *et al*., 2015, Al-Sharqawi

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et al., 2019 and Yassin *et al*., 2020). Strategies to improve the utilization of rice straw are summarized and biological treatments have been investigated, including supplementation with other feed stuffs or components to increase the utilization of rice straw by ruminants (Reddy, 1996; Karunanandaa and Varga, 1995; Shen *et al.*, 1999; Vu *et al.*, 1999; Liu and Ørskov, 2000; Selim *et al*., 2004 and Sheikh *et al*., 2018). The biological treatments providing vitamins and organic acids to support and stimulate the growth of rumen protozoa, and cellulolytic bacteria (Kumar *et al*., 2013, Hristov *et al*., 2013 and Kewan *et al*., 2021).

In order to improve the nutritional value of straw through selective delignification, it may be possible to use fungi and/or their enzymes that break down lingo-celluloses (Jalc, 2002). Fungi are examples of biological treatments that could be used to improve the digestibility of poor-quality forages and eliminate lignin (Khattab, *et al*., 2011; Sharma and Arora, 2015). Exo, Endo-Gluconases, and ß-Glycosidase are sufficient amounts of cellulolytic enzymes produced by fungal treatment of RS with TV (Fadel, 1983). According to Kong *et al.* (2021), *Aspergillus oryzae* Aspergillus niger (AOAN) may provide enzymes to enhance rumen fermentation and improve feed digestibility.

The present study was carried out in order to investigate the impact of a partial replacement of Berseem hay with biologically treated rice straw on the performance of growing sheep.

MATERIALS AND METHODS

The study was conducted at the Experimental Station and Nutrition Laboratory, Animal Production Department, Faculty of Agriculture, Menoufia University (Shebin El-Kom) in compliance with Menoufia University guidelines for dealing with animals in scientific research, with the approval of Ethics Committee. (The Institutional Animal Care and Use Committee- Menoufia University (IACUC) - (Reference No. MUFAG/F/AP/7/22).

Rice straw was chopped at length 2-3cm according to recommendations by (Smail *et al*., 1995) and treated with *Trichoderma viride* (EMCC107), and *Aspergillus fumigatus*. (EMCC:301) which obtained from Microbiology Research Center (MIRCEN), Faculty of Agric., Ain Shams University, Egypt. Facultative fungal strain was chosen according to the studies of Raimbault (1998) and El-Ashry *et al*. (2002).

Twenty seven Barki lambs, seven months old, with live body weight of 33.33±2.21kg were split into three comparable groups (9 lambs /group) according to their live body weight. Daily feed allowance was changed quantitatively according to the change in body weight. Feed was provided twice daily at 9:00 and 16.00 hr. Fresh water was getable at all times. Animals were fed to meet their DM requirements in accordance with (NRC, 1985).

Three rations were prepared with the same roughage: concentrate ratio of 40:60% (R1-Control (60% CFM + 40% berseem hay, R2- 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Trichoderma viride*), R3- 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Aspergillus fumigatus*). Concentrate feed mixture formulation of the experimental rations is presented in Table (1). The chemical composition of the experimental feeds and rations is shown in Table (2).

Feeds and feces samples were carried out in accordance with AOAC (2000). Samples of untreated and treated rice straw were analyzed for fiber fraction according to Van Soest, *et al*., (1991).

Body weights of lambs in a 84-day trial were recorded at the beginning of the experiment and every 14 days through the experimental period. Weighing was done at early morning before the animals were allowed to access feed and water. After completion of the feeding trial, four animals per treatment were used in a metabolism trial. After 21 days as an adapted period feces and urine were collected from the experimental animals. The amount of feces per each animal was measured and recorded daily, mixed, and 10%

representative samples were obtained and immediately frozen at -20°C. Offered feed samples, refusal and feces were all well mixed in and oven dried for 48 hours at 70°C. After being processed through a 1 mm strainer, the dried samples of feeds and feces were stored for analysis. NR: Nutritive ratio = (TDN-DCP)/ DCP. NQI: Nutritive quality index = (CP %) × (DMD %) /100 were calculated according to McDonald *et al*. (1983).

Rumen fermentation parameters (pH, VFA and NH3-N), samples of rumen liquor were obtained at 0, 3 and 6h post feeding using stomach tube. The ruminal pH was determined immediately using the pH meter (Model HI 8424). Free ammonia-N in the rumen samples was measured by the Van Slyke method as described by Ahmed (1976), the volatile fatty acids (VFA) were determined by the steam distillation methods as described by Eadie *et al.* (1967).

Blood serum criteria; total protein, albumin, globulin, urea, creatinine, blood glucose, AST and ALT in were determined using standard kits supplied by Spectrum, Germany.

Immunoglobulin A, immunoglobulin G and Interleukne 2 concentrations in serum were determined using single radio-immunodiffusion methods derived primarily from the works of Fahey and McKelvey (1965) and Mancini *et al.* (1965). These methods are specific for the quantitative determination of individual protein groups in biological fluids.

Table 1: Concentrate feed mixture formulation of the experimental rations.

Ingredients	Experimental rations		
	R1%	R2%	R3%
Yellow corn	50	47	47
Soybean meal	8	11.5	12
Cottonseed meal	17	17	17
Wheat brain	22	21.5	21
Limestone	1.7	1.7	1.7
Min&Vit Mix	0.3	0.3	0.3
Nacl	1	1	1

R1: 60% CFM + 40% berseem hay (Control group). R2:- (Ration2) 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Trichoderma viride*). R3:- Ration3 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Aspergillus fumigatus*).

Table 2 : Chemical composition (%) of the experimental feeds and rations.

Item	Experimental feeds		Experimental rations		
	Clover hay	Rice straw	R1	R2	R3
Dry Matter; DM	**88.02**	**90.12**	**90.45**	**89.32**	**89.15**
Nutrients % on DM basis					
Organic matter; OM	88.3	85.96	85.38	85.84	85.45
Crude protein; CP	12.53	2.65	13.98	14.08	14.02
Crude fiber; CF	32.4	40.12	15.62	17.21	17.10
Ether extract; EE	3.53	2.62	3.22	2.52	2.35
Nitrogen free extract; NFE	39.84	40.57	52.56	52.03	51.98
Ash	11.7	14.04	9.55	10.62	10.85

R1: 60% CFM + 40% berseem hay (Control group). R2:- (Ration2) 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Trichoderma viride*). R3:- Ration3 60%CFM + 40% Roughage (70% berseem hay + 30% rice straw treated with *Aspergillus fumigatus*)

The obtained results were statistically analyzed using Statistical Analytical System (SAS, 2002), Version, 9.3.1 The following statistical model was applied:

Model:- $Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = Parameters under analysis.

μ = Overall mean.

T_i = Treatment effect, (i = 1 …and 3).

eij = Random error.

Differences among means were evaluated using Duncan's (1955) Multiple Range test.

RESULTS AND DISCUSSION

Effect of fungal treatment on chemical composition and Cell wall constituents of rice straw:

Effect on chemical composition

The chemical composition of the experimental rations is shown in Table (3). Untreated RS has significantly (P < 0.05) lower OM content (83.77%), CP (3.15%), and NFE (44.02%) than the treated RS with TV (85.95, 6.05, and 47.1%) and treated RS with AF (being 85.03, 5.09 and 45.24%), respectively. The other contents of EE and ash were almost relative in all treatments; while the URS contains significantly (P< 0.05) higher CF 35.45% than that treated with TV (31.55%) or that treated with AF (33.52%). Treating RS with TV increased CP content significantly (P<0.05) where the level reaching of 6.05% (TV) which was almost double the content of untreated rice straw (Table 3). Rice straw has a low crude protein (CP), high crude fiber (CF) content and low digestion coefficients. Several experiments have been carried out on non-protein nitrogen treatments to increase its protein content (Shoukry, 1992; El-Ashry, *et al.,* 2002, Hanafi *et al.,* 2012; Jaleta *et al.,* 2013 and Oladosu *et al.,* 2016). Study of Hanafi, *et al*. (2012) has focused on increasing the nutritive value of rice straw through physical, chemical, or biological treatments, fungal treatment of RS or corn stalk by TV reduced the contents of OM, CF, NFE, ADF, ADL, cellulose, and hemicellulose. Abdel-Azim *et al*. (2011) noted that treating with TV increased OM content of corn stalk than RS under the same conditions, while contents of CP, EE, and ash increased than those of untreated one. According to El-Bordeny *et al*. (2015), rice straw that had been biologically treated with *Trichoderma reesei* or *Trichoderma viride* had a low DM content. Moreover the reduction of DM content in rice straw by (24.13%-5.35%), in barley straw by (28.8%-5.8%), in rice bran straw by (22.1%-5.03%), and in wheat straw by (23%-1.3%) in bacterial treated groups. *T. viride* has the ability to quickly proliferate and take part in the degradation reaction of rice straw (Belal, 2013). They suggested that this might be because fungi increased adaptation to anthropogenic substrates and wide range of resistance to the factors that impact fungi during recycling processes. Mahrous *et al*. (2005) indicated that the CP of RS increased using biological treatments, while the DM, CF, NFE, NDF, ADF, ADL and cellulose contents decreased (P < 0.05) than the control group.

Effect on cell wall constituents

The data of fiber fractions for the experimental diets of the present study are shown in Table (3). These results revealed that the content of NDF significantly (P < 0.05) declined with treated rice straw via either TV or AF by 10.37% and 7.21% respectively compared with URS**.**The same trend shows with Acid detergent fiber (ADF) which was 56.22% for the untreated rice straw than treated rice straw with either TV (50.17%) or AF (51.64%). The same pattern for contents of cellulose, hemicellulose, and ADL was shown as in NDF and ADF. Where the cellulose values were significantly (P <0.05) decreased by 8.36 and 5.98% in treated RS with TV and AF than those in URS, while hemicellulose values decreased (P <0.05) significantly by 9.50 and 5.25 % in the same treatments. At the same time values of ADL decreased (P < 0.05) significantly by 20.54 and 16.85% in treated RS via TV or AF, respectively; compared to URS shows changing values of the cell wall constituents of rice straw as affected by fungal treatments. These data are close to those reported by (El-Ashry, *et al*., 2002, Hanafi *et al*., 2012; Jaleta *et al*., 2013 and Oladosu *et al*., 2016). Abdel-Azim *et al*. (2011) and Belal, (2013) reported improved chemical composition of rice straw treated with *T. viride* through decreasing CF by 14.69 % and increasing CP content by 7.28 %.

The low digestion of roughage residues is caused by their high lignin and cellulose content (Abdel-Azim *et al.*, 2011). Accordingly, many studies were carried out to determine the nutritional value of crop residues and straws by Khattab *et al.* (2011) and Kholif *et al.* (2015). They came to the conclusion that feeding on these roughage residues would not even meet maintenance energy requirements without treatment or nutritional supplementation.

Effect of fungal treatment on animal growth performance:

Results of change in the body weight of sheep are present in Table (4). The initial body weight was almost equal in all groups (avg. 33.3kg). Animal body weights gradually increased with experimental periods, particularly after 2 weeks of being experimental, where the average body weight was 34.8, 35.25, and 34.89kg for groups feed URS, TV-treated and AF-treated, respectively. Values of body weight are in the same range reported by Khattab *et al*., 2011; Kholif *et al*., 2015. The increase in body weight was not noticeable until the sixth week, after that, increases began to appear (P <0.05) significantly in the experimental animals' groups that were fed the TV-treated and AF-treated compared to that fed URS. Body weight was 39.5, 41, and 40.98kg at week 8 for sheep fed the experimental diets containing URS, TV-treated and AF-treated, respectively. The respective values at week 10 were 41.54, 43.38 and 43.19kg. The final body weight was 43.45, 45.5, and 45.35kg for groups fed URS, TV-treated and AF-treated, respectively; while the total gain was 10.18, 12.07, and 12.14kg for the respective groups. These data are close to those reported by Abdel-Azim *et al*. (2011). Feeding the experimental diets treated with TV improved the growth rate by 18.6% opposite 19.25% for the group that fed AF-treated. The average daily gain ADG was increased (P <0.05) significantly with fed treated RS with either TV or AF compared to a group fed URS. The overall mean values of ADG were 121, 143.67, and 144.44g/d, for URS, TV, and AF, respectively. These data are close to those reported by Jaleta *et al*. (2013) and Oladosu *et al*. (2016). The biological treatment impact on the effectiveness of the microorganisms in the rumen may be the cause of the improvement in the daily weight gain (DWG) of experimental animals fed on biologically treated-RS. The digestibility of organic matter directly reflects this effect (Abdel-Azim *et al.,* 2011).

Alwaeli (2016) argues that improved digestion of biological treatments improves feed conversion. Moreover, Gado *et al*. (2007) reported that a significant increase in the concentration of cellulase enzyme in biologically treated materials improves the digestibility of the feed provided and thus reflects on the average body weight gain. Omer *et al*. (2012) demonstrated that biologically treated corn stalks (*Trichoderma ressi*) can entirely replace clover hay in the diet of growing sheep. This was demonstrated by a positive increase in DM intake and an improvement in the digestibility of all nutrients with greater ADG.

Table 3: Chemical composition and Cell wall constituents of rice straw as affected by fungal treatment

Chemical composition on DM basis%					
Nutrients	**Treatments**			SEM	*P*. value
	URS	TV	AF		
Dry matter, DM	91.23	89.16	89.13	0.85	0.580
Organic matter, OM	83.77^{b}	85.95^{a}	85.03^{a}	0.34	0.003
Crude protein, CP	3.15^{c}	6.05^{a}	5.09^{b}	0.43	<0.001
Crude fiber, CF	35.45^{c}	31.55^{a}	33.52^{b}	0.59	0.001
Ether extract, EE	1.15	1.24	1.18	0.51	0.814
Nitrogen free extract, NFE	44.02^{b}	47.10^{a}	45.24^{b}	0.46	<0.001
Ash	16.23	15.05	14.77	0.56	0.587
Cell wall constituents on DM basis%					
Neutral detergent fiber, NDF	82.33^{c}	73.79^{a}	76.38^{b}	1.29	0.001
Acid detergent fiber, ADF	56.22^{b}	50.17^{a}	51.64^{a}	0.94	0.001
Cellulose	45.12^{c}	41.35^{a}	42.42^{b}	0.58	0.001
Hemicellulose	26.11^{c}	23.63^{a}	24.74^{b}	0.39	0.002
Acid detergent lignin, ADL	11.10^{b}	8.82^{a}	9.23^{a}	0.38	0.004

^{a,b,c} means within each raw with different superscript differ significantly. URS, untreated rice straw, TV, treated rice straw with *Trichoderma viride*; AF, treated rice straw with *Aspergillus fumigatus*.SEM, standard error of means

Table 4: Growth performance as affected by the partial replacement of clover hay with fungal treated rice straw

Weeks	**Treatments**			**SEM**	***P*. value**
	C	**TV30%**	**AF30%**		
Initial BW (kg)	33.27	33.42	33.21	0.28	0.956
2 weeks	34.80	35.15	34.89	0.27	0.876
4 weeks	36.36	36.75	36.45	0.29	0.859
6 weeks	37.93	38.33	38.05	0.25	0.812
8 weeks	39.50^{b}	41.00^{a}	40.98^{a}	0.17	0.001
10 weeks	41.54^{b}	43.38^{a}	43.19^{a}	0.19	0.001
Final BW (kg)	43.45^{b}	45.50^{a}	45.35^{a}	0.21	< 0.001
Total weight gain (kg)	10.18^{b}	12.07^{a}	12.14^{a}	0.28	0.002
ADG (g)	121.00^{b}	143.67^{a}	144.44^{a}	3.35	0.002

C: control diet 60%CFM plus 40%B.hay. TV.30%: 60%CFM plus 40% Roughage (70% B.hay plus 30% rice straw treated with Trichoderma viride). AF,30%: 60%CFM plus 40% Roughage (70% B.hay plus 30% rice straw treated with Aspergillus fumigatus). BW, Body weight; ADG, Average daily gain
SEM, standard error of means.
^{a,b} means within each row with different superscript differ significantly

Effect of fungal treatment on nutrient digestibility nutritive value and nitrogen balance

Effect on Nutrient digestibility

Results given in Table (5) show the apparent digestion coefficients of nutrients (mean ±SE) in the different experimental diets. No significant differences were found in apparent digestion coefficients of DM among experimental diets which stood at 69.13, 69.81

and 70.10% for the diets C, TV, and AF, respectively. Groups fed biological treatment of RS via either TV or AF, improved the apparent digestion coefficients of CP by about 3-4% compared to URS, and the values were 67.15, 71.76, and 72.95% for C, TV, and AF, respectively.

Data in Table (5) revealed that apparent digestion coefficients of CF for diets containing treated-RS significantly improved (P<0.01) by about 8%, and the values were 51.35, 55.65, and 54.55% for the same respective diets. Generally, the digestibility of NFE and EE was almost similar in all experimental treatments being 75.26, 75.89, and 75.88% for NFE and 73.25, 73.77, and 74.11% for EE. Many past studies suggested that biological treatment work better at enhancing nutrient digestibility than chemical ones (Kabirifard *et al*., 2007; Khattab *et al*., 2009; Abdel-Aziz *et al*., 2014). Fungi are examples of biological treatments that could be employed to eliminate lignin and improve the digestibility of poor quality forage (Khattab, *et al*., 2011; Sharma and Arora, 2015). The biological effect of the fungus may have improved the environment in the digestive tract, which would explain the improvement in digestibility. Mahrous *et al.* (2005) investigated the effects of biological treatments (ZAD, fungus, and ZAD plus fungus) for rice straw on digestibility, nutritional value, and nitrogen balance. They found that, in contrast to the control group, fungal treatment enhanced the crude protein content of rice straw while lowering the amounts of dry matter, crude fiber, NFE, NDF, ADF, and cellulose.

Effect on digestibility of cell wall constitutes

The results in Table (5) showed the effect of different experimental diets on apparent digestion coefficients of cell wall components. Apparent digestion coefficients of NDF showed differences (P< 0.01) significant which were 50.55, 53.48 1and 52.18 % for the diets C, TV, and AF, respectively. Differences were statistically (P< 0.01) significant for apparent digestion coefficients of ADF as shown in with feeding sheep diet containing URS, RS treated with TV, and RS treated with AF, where the values were 52.20, 54.26, and 53.42 % for the same respective diets. The current study revealed that feeding sheep on feed containing RS biologically treated improved ADF digestibility by about 4%.

Data in Table (5) revealed that apparent digestion coefficients of cellulose were 47.88, 50.84, and 48.46% when sheep fed diets containing URS, RS treated with TV, and RS treated with AF. The differences were statistically (P< 0.01) significant among different experimental groups. Digestibility of hemicellulose was almost similar to digestion coefficients of cellulose in all experimental treatments being 58.63, 58.96, and 59.02% for the experimental diets containing URS, RS treated with TV, and RS treated with AF.

Abo-Donia *et al*. (2005) and Abedo *et al*. (2009) attribute the improvement in the digestion of dietary fiber to the improvements in the gastrointestinal environment due to the biological effect of the fungi. Mahrous *et al*., (2005) reported that bacterial colonization affects cellulose and voluntary intake of treated straws. The soaking of the straw causes an explosion of plant cells, which leads to a significant increase in the coefficient of fiber digestion in-vivo, and confirms that fungicides using fungi and their enzymes improve the quality of rice straw (Kabirifard *et al*., 2007; and Khattab *et al.*, 2009).

Hanafi *et al*. (2012) reported that solid-state fermentation (SSF) as a typical method for the production of microbial metabolites requires further development and that the practical application of these therapies is still constrained by safety issues, financial constraints, and the possibility of unfavourable environmental effects. To increase the digestibility of crop residues as animal feed, Abo-Donia *et al.* (2005), Sazzad and Sabita (2008), Abedo *et al.* (2009) and Abdel-Aziz *et al.* (2014) concluded that the binding between cellulose, hemicellulose, and lignin must be released or the compact nature of these tissues modified so that lignified tissue may separate from nonlignified tissue. There have been numerous attempts to accomplish this through mechanical, chemical, or biological treatments. Biological treatments are one of the techniques that have received much interest for their use in improving the accessibility of the cellulosic fractions of crop residues, thus improving their digestibility and nutritional value, so they have attracted wide interest among researchers (Zhang *et al*., 2007; Yu *et al*., 2009; Mahesh and Mohini, 2013 and Asmare, 2020).

Effect on Nutritive value:

The nutritive values of the experimental diets are presented in Table (5). The results indicated that the nutritive values of diets containing the tested ingredients were not significantly (P< 0.01) different. All treatments had comparable nutritive values, where the TDN values were (63.00, 63.83, and 63.08%), while the values of DCP were (10.11, 10.57, and 10.39%) for C, TV, and AF, respectively. Regarding the nutritive ratios, no significant differences were found which existed at 5.26, 5.07, and 5.10 for dietary groups C, TV, and AF-treated RS. The respective values of the nutritive quality index (NQI) were 9.66, 9.83, and 9.83 for the experimental groups, respectively. In several studies, it was indicated that different treatments or the supplementary of additional required nutrients to the animal ration could increase the feeding value of rice straw. The results of biological treatments that have been looked into, including the supplement of other feed stuff or components to increase the utilization of rice straw by ruminants and provide a summary of strategies to improve the utilization (Reddy, 1996; Karunanandaa *et al*., 1995; Shen *et al.*, 1999; Vu *et al.*, 1999; Liu and Ørskov, 2000; Selim *et al.,* 2004 Sheikh *et al*., 2018).

Abdel-Azim *et al.* (2011) briefed that treatment of RS and corn stalks with TV, improved nutrition values, which was reflected in increased intake, N-balance, and growth rate in cross lambs. Moreover, According to Al-Samaraae and Alwaeli (2016), the application of fungal treatments enhanced the feeding value of agricultural byproducts. Furthermore, the biological treatments aid to break the bond between lignin and cellulose and raise free cellulose to facilitate easier use by ruminants (Mahesh and Mohini, 2013). As a result, *Trichoderma harzianum* was researched for use in biological treatment to dissolve the bond between lignin and cellulose (Alwaeli, 2016). Improving the nutritional value of agricultural residues leads to an increase in the animals' benefits from them, and according to (Morteza *et al*., 2010) it contributes to improving the carcass quality. Abdel-Azim *et al*. (2011) reported that low DM digestibility and low protein available in RS cause low nutritional values. Due to the low nutritional value of this highly lignified material, feeding rice straw to ruminants as sole feed does not provide sufficient nutrients for high levels of production (Morteza *et al.*, 2010).

Van Soest (2006) summarized the main deficiencies of RS that affect its value as a ruminant feed as being its high lignin content and conversion to silicates, delayed and limited degradation of carbohydrates, and low nitrogen content. According to Kholif *et al*. (2005) and Mahrous (2005), roughages treated with fungi had higher TDN levels and DCP compared to untreated materials, and they also had better (P < 0.05) nutrient digestibility.

Effect on nitrogen balance:

Nitrogen balance data for sheep fed the experimental diets (Table 5) showed that NI was nearly convergent in all groups with values of 20.03, 19.55, and 19.3 g/day for the C, TV, and AF treated groups, respectively. The respective values of faecal nitrogen (FN) showed significant (P < 0.05) differences with values of 6.58, 5.52, and 5.22 g/day, while urinary nitrogen was close in all groups with 9.41, 9.05, and 9.05 g/day for the C, TV, and AF treated groups, respectively. The NB values were 4.03, 4.98, and 5.03 g/day for the three experimental groups, respectively. It was evident that NB (Table 5) took the same trend of the average daily gain (121.00, 143.67, and 144.44 g/day) which appear in table 4. The biological value (BV) of the dietary protein was calculated (NB/NI) and presented in Table (5), which was significantly (P< 0.01) higher for groups AF (35.66%) and TV (35.48%) and lowest for the control group (30.00%). Accordingly, the relationship between NB and ADG was determined and the regression coefficient was positively and highly significant (r2 = 0.81). The results indicated that the incorporation of RS treated with AF or TV fungi into sheep rations leads to an improvement in digestion coefficients and nutritive values compared to using untreated RS. According to Mahrous *et al*. (2005), recommended that biological treatment of RS using a ZAD solution or a fungus (*Pleurotus osteratus*) improved the nutritive value and NB.

Table 5: Nutrient digestibility, nutritive value and nitrogen balance of sheep as affected by the partial replacement of clover hay with fungal treated rice straw

Items	Treatments			SEM	*P.* value
	C	TV30%	AF30%		
Nutrient digestibility (%)					
Dry matter, DM	69.13	69.81	70.10	0.45	0.722
Crude protein, CP	67.15[c]	71.76[b]	72.95[a]	0.42	0.001
Crude fiber, CF	51.35[b]	55.65[a]	54.55[a]	0.75	0.019
Nitrogen free extract, NFE	75.26	75.89	75.88	0.35	0.75
Ether extract, EE	73.25	73.77	74.11	0.30	0.566
Digestibility of cell wall constitutes (%)					
Neutral detergent fiber, NDF	50.55[c]	53.48[a]	52.18[b]	0.44	0.001
Acid detergent fiber, ADF	52.20[c]	54.26[a]	53.42[b]	0.31	0.001
Cellulose	47.88[b]	50.84[a]	48.46[b]	0.47	0.001
Hemicellulose	58.63	58.96	59.02	0.33	0.90
Nutritive value (%)					
TDN	63.00	63.83	63.08	0.33	0.590
DCP	10.11	10.57	10.39	0.26	0.807
NR	5.26	5.07	5.10	0.18	0.92
NQI	9.66	9.83	9.83	0.66	0.555
Nitrogen balance (g/d)					
Nitrogen intake, NI	20.03	19.55	19.30	0.25	0.544
Fecal nitrogen, FN	6.58[b]	5.52[a]	5.22[a]	0.23	0.007
Urinary nitrogen, UN	9.41	9.05	9.05	0.12	0.434
Nitrogen balance, NB	4.03[b]	4.98[a]	5.03[a]	0.18	0.011
Biological value, BV%	30.00[b]	35.48[a]	35.66[a]	1.02	0.006

C: control diet 60%CFM plus 40%B. hay. TV.30%: 60%CFM plus 40% Roughage (70% B. hay plus 30% rice straw treated with *Trichoderma viride)*. AF, 30%: 60%CFM plus 40% Roughage (70% B. hay plus 30% rice straw treated with *Aspergillus fumigatus*).TDN: total digestible nutrients. DCP: digestible crude protein. NR: Nutritive ratio = (TDN-DCP)/ DCP. NQI: Nutritive quality index = (CP %) × (DMD %) /100. SEM: standard error of means

[a ,b and c] means within each row with different superscript differ significantly.

Effect of fungal treatment on rumen fermentation:

The conducted treatments shown in Table (6) revealed that treating rice straw with either TV or AF fungus did not affect the rumen pH values at any time of feeding. Total volatile fatty acids: The effect of treating rice straw with TV or AF fungus on volatile fatty acid in the rumen is presented in Table (6). No significant differences were reported at zero time for the C, TV, and AF treated groups (8.12, 8.23, and 8.32 meq/dl, respectively). The values of VFA production raised (P< 0.001) significantly three hours after feeding from 10.12 in the C diet to 12.98 for TV and 12.35 meq/dl in AF. However, the values of VFA produced in the rumen of sheep after six hours of feeding were similar, and the results were virtually close for all groups (12.96, 12.92, and 12.38 meq/dI for control, TV-treated, and AF-treated rations, respectively).

The effect of treating RS with either TV or AF fungus on ammonia-N concentration in the rumen is presented in Table (6). No significant differences were found at zero time for NH3-N concentrations which were 14.88, 15.11, and 15.02 mg/dl for control, TV-treated, and AF-treated rations, respectively. At three hours from feeding NH3-N concentration significantly (P< 0.005) increased from 16.25 in the C diet to 18.65 for TV and 18.39mg/dl for AF. While the NH3-N concentration at six hours after feeding was driving the same trend as the concentrations at 3 hours, which reached 18.38, 20.05, and 19.95 mg/dL for the control, TV-treated, and TV-treated rations, respectively. These results support the suggestion that the NH3-N concentration decreases when feeding on untreated RS as a result of containing lower nutritional values with lower DM digestibility and lower protein content (Abdel-Azim *et al*., 2011).

As a result of the low nutritional value of this highly lignified material, Liu and Ørskov (2000), Abo-Donia *et al*. (2005), and Abedo *et al*. (2009) concluded that feeding ruminants only rice straw is insufficient to maintain high production levels. Additionally, the main deficiencies of RS, which reduce its usefulness as ruminant feed, are its high level of lignification and silicification, sluggish and limited ruminal degradation of the carbohydrates, and low nitrogen content (Van Soest, 2006).

Table 6: Ruminal parameters in sheep as affected by the partial replacement of clover hay with fungal treated rice straw

Incubation hour	**Treatments**			**SEM**	***P.* value**
	C	**TV30%**	**AF30%**		
Rumen liquor pH					
0	6.22	6.39	6.70	0.18	0.616
3	6.58	6.48	6.77	0.13	0.706
6	6.29	6.40	6.68	0.16	0.669
NH_3-N, mg/dl					
0	14.88	15.11	15.02	0.16	0.875
3	16.25^b	18.65^a	18.39^a	0.42	0.005
6	18.38^b	20.05^a	19.95^a	0.29	0.003
VFA, meq/dl					
0	8.12	8.23	8.32	0.17	0.915
3	10.12^b	12.98^a	12.35^a	0.46	0.001
6	12.96	12.92	12.38	0.23	0.576

control diet 60%CFM plus 40%B.hay. TV.30%: 60%CFM plus 40% Roughage (70% B.hay plus 30% rice straw treated with Trichoderma viride*)*. AF,30%: 60%CFM plus 40% Roughage (70% B.hay plus 30% rice straw treated with Aspergillus fumigatus). SEM: standard error of means

$^{a,b \text{ and } c}$ means within each raw with different superscript differ significantly

In a study (Jahromi *et al*. 2010) on the treatment of RS using *Aspergillus niger* (K8) with or without the addition of nitrogen source (urea), the protein in the treated straw was significantly ($P < 0.01$) increased after fermentation. The results showed that fermentation of RS using *A. niger* significantly ($P < 0.01$) decreased the production of acetate and propionate and the production of total volatile fatty acids (VFA). The research concluded that solid-state fermentation of rice straw with A. niger decreases the lignocellulose content, but has a negative impact on microbial activity in the rumen ecosystem. This is likely because of the anti-A. niger activity, or other intermediate products of fermentation, on rumen organisms. Aziz (2014 and 2019) found that ruminal pH and total VFA concentrations of adult sheep were improved with a ration containing SBP treated with *T. viride*, *S. cerevisiae* and C. cellulase more than untreated SBP and control. Aziz (2020) reported that biological treatments for Sugar beet pulp (SBP) significantly increased ($P \leq 0.01$) ruminal pH values, total ruminal VFA, microbial protein, ammonia nitrogen, non-protein nitrogen, true protein, total nitrogen, and ruminal protozoa numbers.

Effect of fungal treatment on blood biochemical and immunity status

Effect on blood biochemical

Blood parameters of sheep as affected by the replacement with fungus treated straw are presented in Table (7). Generally, all values were within the normal values of blood characteristics of sheep. Total protein (g/dl) significantly ($P<0.02$) increased from 6.03 in group C to 6.99 and 6.88in groups TV and AF. Albumin values were 3.02, 3.95 and 3.8g/dl for the same respective order; differences were significant ($P<0.03$). However, values of globulin were equal in all groups (3.01-3.08). Glucose concentration (mg/dl) was 67.71, 72.62 and 71.33 for the experimental animals of C, TV and AF, respectively. Differences were statistically significant ($P<0.01$). All other criteria were almost the same in animals without any effect of fungus treatment. Values of plasma urea, creatinine, AST and ALT were within the normal range indicating that fungus treatment did not have any harmful effect on liver and kidney function.

Mahrous *et al.*, (2005) investigated the impact of rice straw biological treatments on blood parameters. There were no significant differences ($P < 0.05$) between any of the treated rice straw groups and the untreated rice straw in terms of plasma urea, total protein, albumin, globulin, GOT, and GPT. El-Marakby (2003) concluded that feeding lambs wheat straw treated with *A. bosporus* spawning had no significant effect on plasma total protein levels. While lambs fed treated wheat straw generally had insignificant higher plasma globulin concentrations than lambs fed the control ration. This might be as a result of enhanced albumin synthesis in the liver brought on by diets containing treated wheat straw. He also pointed non-significant differences between treatments in plasma AST and ALT for lambs fed biologically treated rice straw compared with control ration. Kholif *et al.* (2005) and Allam *et al.* (2006) reported that biological treatments increased serum total protein, urea, glucose and aspartate aminotransferase. They added that the biological treatment had no effect on the serum globulin. Aziz (2009) illustrated that blood concentrations of total protein, albumin, globulin, and creatinine in lambs fed by-products from olive trees treated with *T. viride* or *S. cerevisiae* were higher than those in controls, however they were lower for concentrations of urea, AST, and ALT. Rivero *et al.* (2012) noted that the administration of enzymes and *S. babylonica* extract to lambs had no detrimental effect on the health of the lambs and had no influence on red blood cells, haematocrit, haemoglobin, white blood cells, segmented neutrophil, lymphocytes, or plasma protein levels of the lambs.

Muhamad (2012) reported that serum urea concentration was decreased (P≤0.01) by adding yeast culture to ration of lambs, while GOT was increased and GPT was decreased in comparison with the other treatments. Aziz (2020) reported that R (3): CFM contained Sugar beet pulp (SBP) treated with Saccharomyces cerevisiae + berseem hay. R (4): CFM contained SBP treated with Trichoderma viride + berseem hay. And R (5): CFM contained SBP treated with Cellulomonas cellulasea + berseem hay increased the concentrations of blood serum glucose, total proteins, albumin, creatinine, urea, GOT, GPT concentrations comparing with untreated SBP.

Effect on immune status

Values of immune status of sheep as affected by the replacement with fungus treated rice straw are presented in Table (7). No significant effects were reported for all values of immune system indicating that treating rice straw with fungus (TV or AF) did not have any negative effect on the immune system. Modulation of the immune system is the important beneficial health attribute of probiotic microorganism.

They have been found to enhance immune responses by proliferation of T-cell and B-cell, cytokine, immunoglobulin (IgA, IgA) production etc., which directly and indirectly help in the prevention of certain diseases like atopic eczema, AIDS, allergy (a hypersensitive reaction), etc. (Kimoto *et al.*, 2007). Enhancing immunity in the elderly with probiotics would be especially beneficial because immune function diminishes with age (Gill and Rutherfurd, 2001).

Table 7: Blood criteria of sheep as affected by the partial replacement of clover hay with fungal treated rice straw

Parameters	Experimental diets			SEM	*P*. value
	Control	TV30%	AF30%		
Total protein (g/dl)	6.03^{b}	6.99^{a}	6.88^{a}	0.16	0.002
Albumin (g/dl)	3.02^{b}	3.95^{a}	3.80^{a}	0.16	0.003
Globulin (g/dl)	3.01	3.04	3.08	0.31	0.659
Glucose (mg/dl)	67.71^{c}	72.62^{a}	71.33^{b}	0.74	0.001
Urea(mg/dl)	28.65	30.12	30.10	0.85	0.773
Creatinine (mg/dl)	1.21	1.23	1.29	0.12	0.966
AST U/L	106.11	107.02	106.21	0.75	0.893
ALT U/L	117.25	118.29	119.38	0.78	0.513
IgA, mg/dl	28.15	30.22	28.43	0.86	0.633
IgG, mg/dl	128.55	129.67	128.98	0.81	0.884
Interleukin 2 (Pg/ml)	88.14	85.45	88.32	0.92	0.418

C: control diet 60%CFM plus 40%B.hay. TV.30%: 60%CFM plus 40% Roughage (70% B.hay plus 30% rice straw treated with Trichoderma viride). AF,30%: 60%CFM plus 40% Roughage (70% B.hay plus 30% rice straw treated with Aspergillus fumigatus). AST, Aspartate transaminase; ALT; Alanine transaminase. SEM: standard error of means

$^{a,b\ and\ c}$ means within each raw with different superscript differ significantly

CONCLUSION

It could by conclude that fungal treatment of rice straw with Trichoderma viride and Aspergillus fumigatus increased CP content up to 6.05% and 5.09% which was almost nearly double the content of untreated rice straw; this treatments decreased NDF and ADF by about 7 to 10%, and improved CF digestibility by about 8%. The biological value of the dietary protein was higher for treated groups and lowest for control group. Rice straw chemical composition could improve by fungal treatment which also improved nutrients digestibility, nitrogen balance and growth performance of growing Barki sheep without negative effect on rumen fermentations, blood biochemical characteristics and immunity status. Fungal treated rice straw could replace up to 30% of clover hay in sheep rations**.**

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تأثير الإستبدال الجزئى لدريس البرسيم بقش الأرز المعامل بالفطريات على أداء النمو، معامل الهضم ، تخمرات الكرش ، كيمياء الدم والحالة المناعية للاغنام البرقى

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الملخص العربى

أجريت هذه الدراسة بمزرعة كلية الزراعة ومعمل تغذية الحيوان بقسم الإنتاج الحيوانى بكلية الزراعة جامعة المنوفية- بهدف دراسة تأثير المعاملة بالفطريات على التركيب الكيماوى لقش الأرز ومن ثم دراسة تأثيرالاستبدال الجزئى لدريس البرسيم بقش الأرز المعالج بالفطريات في علائق الأغنام على أداء النمو، الهضم، تخمرات الكرش ، قياسات الدم والحالة المناعية للاغنام البرقى . تم استخدام سبعة وعشرين ذكر من الاغنام البرقيً ، بعمر سبعة أشهر ومتوسط وزن يبلغ ٣٣,٣٣ ± ٢,٢١ كجم فى تجربة لمدة ١٢ أسبوع . قسمت الحيوانات إلى ثلاث مجموعات (٩ حيوانات/ معاملة) لثلاثة علائق تجريبية بنفس نسبة المركز : المالئ (٦٠: ٤٠٪) : مجموعة المقارنة ؛ ٦٠٪ علف مركز + ٤٠ % دريس برسيم ومجموعة المعاملة الأولى (TV) ؛ ٦٠٪ علف مركز +٤٠٪ مالئ (٧٠٪ دريس برسيم + ٣٠٪ قش أرز معامل بفطر *Trichoderma viride*) ومجموعة المعاملة الثانية (AF)؛ ٦٠٪ علف مركز +٤٠٪ مالئ (٧٠٪ دريس برسيم + ٣٠٪ قش أرز معامل بفطر *Aspergillus fumigatus*.) وتم تسجيل أوزن الجسم للحيوانات في بداية التجربة وكل أسبوعين حتى الانتهاء من تجربة النمو ، بعد انتهاء تجربة النمو تم استخدام أربعة حيوانات لكل معاملة في تجربة هضم .أشارت اهم النتائج الى:

- أدت المعاملة بالفطريات الى ارتفاع محتوى قش الأرز من المادة الجافة والبروتين الخام والكربوهيدرات الذائبة مقارنة بقش الأرز غير المعامل. كما احتوى قش الأرز غير المعامل على نسبة أكبر من الالياف الخام ٣٥,٤٥٪ مقارنة ب TV (٣١,٥٥٪) و AF (33.52) و أدت معاملة قش الأرز ب *Trichoderma viride* إلى زيادة محتواه من البروتين الخام إلى ٦,٠٥٪ وهو ما يقرب من ضعف محتوى قش الأرز غير المعامل و بلغت قيم الألياف المتعادلة (NDF) ٨٢,٣٣ و ٧٣,٧٩ و ٧٦,٣٨٪ للقش الغير معامل و المعامل بTV و المعامل ب AF على التوالي حيث انخفضت نسبة الألياف المتعادلة نتيجة معاملة قش الأرز بالفطريات بحوالي ٧ إلى ١٠٪. واتبعت ألياف المذيبات الحمضية (ADF) نفس الاتجاه حيث سجلت قيما أعلى (٥٦,٢٢٪) لقش الأرز غير المعامل مقارنة بقش الأرز المعامل بالفطريات والذي كان متساويًا تقريبًا (٥٠,١٧ و ٥١,٦٤٪) في كلا المعاملتين.
- لم تظهر فروق معنوية فى أوزان جسم الحيوانات بين المجموعات التجريبية حتى الأسبوع السادس ؛ ثم بدأت الزيادات تصل إلى المستوى المعنوي عند الأسبوع الثامن. كان وزن الجسم النهائي ٤٣,٤٥ و ٤٥,٥ و ٤٥,٣٥ كجم عند الاسبوع الثانى عشر . وبلغ إجمالي الزيادة الكلية فى الوزن ١٠,١٨ و ١٢,٠٧ و ١٢,١٤ كجم وبلغت قيم معدل النمو اليومي ١٢١ و ١٤٣,٦٧ و ١٤٤,٤٤ جم / يوم للمجموعة المقارنة ومجموعة TV ومجموعة AFعلى الترتيب.
- بلغت قيم معاملات هضم المادة الجافة لمجموعة المقارنة و مجموعة TV ومجموعة AF ٦٩,١٣ ٦٩,٨١ و ٧٠,١٠٪ على التوالي ومعاملات هضم للبروتين ٧٢,٣٢ و ٧٥,٠٥ و ٧٤,١٢٪ وأيضا معاملات هضم الألياف ٥١,٣٥ و ٥٥,٦٥ و ٥٤,٥٥٪ لنفس المجموعات على الترتيب حيث أدت المعاملات إلى تحسين هضم الالياف الخام بحوالي ٨٪. وكانت معاملات هضم الالياف المتعادلة NDF ٥٠,٥٥ و ٥٣,٤٨ و ٥٢,١٨٪ و معاملات الهضم الظاهرية للالياف الحامضية -ADF ٥٢,٢ و ٥٤,٢٦ و ٥٣,٤٢٪ للعلائق المقارنة و TV و AF على التوالي.
- لم تختلف كثيرا القيمة الغذائية للعلائق التجريبية وكانت لجميع المعاملات قيم غذائية متشابهة ، حيث بلغت ٦٣,٠٠ و ٦٣,٨٣ و ٦٣,٠٨٪ لـ TDN و ١٠,١١ و ١٠,٥٧ و ١٠,٣٩٪ لـ DCP الجافة لمجموعة المقارنة و مجموعة TVومجموعة AF على التوالي.

- قيم ميزان النتيروجين NB كانت ٤,٠٣ و ٤,٩٨ و ٥,٠٣ جم / يوم لمجموعة المقارنة و مجموعة AF ومجموعة TV على التوالي والقيمة البيولوجية للبروتين الغذائي كانت أعلى لمجموعتيAF ٣٥,٦٦٪ و ٣٥,٤٨ ٪ TV وأقل للمجموعة المقارنة (٣٠,٠٠٪).
- لم تؤثر معاملة قش الأرز بفطر TV أو AF على قيم pH الكرش في أي وقت بعد التغذية , فى حين بعد ثلاث ساعات من التغذية ارتفع إنتاج الاحماض الدهنية الطيارة بالكرش من ١٠,١٢ في المجموعة المقارنة إلى ١٢,٩٨ لمجموعة TV و ١٢,٣٥meq / dl مجموعة AF.و بعد ست ساعات من التغذية كان إنتاج الاحماض الدهنية الطيارة متساويًا تقريبًا لجميع المجموعات التجريبية. كما زاد تركيز امونيا الكرش من ١٦,٢٥ في المجموعة المقارنة C إلى ١٨,٦٥ في مجموعة ال TV و ١٨,٣٩ مجم / ديسيلتر فى مجموعة ال AF بعد ثلاث ساعات من التغذية. واتخذ إنتاج الأمونيا اتجاه مماثل بقيم ١٨,٣٨ و ٢٠,٠٥ و ١٩,٩٥ ملجم / ديسيلتر للمجموعات التجريبية على التوالي بعد ست ساعات من التغذية
- بشكل عام ، كانت جميع قيم مكونات الدم ضمن القيم الطبيعية لخصائص دم الأغنام. ازداد البروتين الكلي من ٦,٠٣ في المجموعة المقارنة إلى ٦,٩٩ و ٦,٨٨ في مجموعتي TV و AF. كانت قيم الألبومين ٣,٠٢ و ٣,٩٥ و ٣,٨ جم / ديسيلتر لنفس الترتيب . قياسات المناعة كانت فى حدودها الطبيعية ولم تختلف معنويا بين المجموعات التجريبية الثلاث.