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Productive and Physiological Response of Ewe-Lambs Fed Ration Containing Bio-Upgraded Rice Straw

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ABSTRACT
This study aimed to investigate effects of inclusion biologically treated rice straw with *Trichoderma viride* or *Trichoderma reesei* in ewe-lambs ration on its productive and some physiological response. Thirty crossbred (Rahmany X Romanof) ewe-lambs (three months old and 17±1.13 kg body weight) were assigned randomly to three groups (10 animals each). The first group was fed ration contained 55% concentrate feed mixture and 22.5% clover hay plus 22.5% Untreated Rice Straw (URS), group two and three were fed rations contained rice straw treated with *T. reesei* (TRSR) or *T. viride* (TRSV), respectively. No significant differences were observed in nutrients digestibility, Total Digestible Nutrients (TDN), nitrogen retention and nitrogen utilization efficiency among the experimental groups. Treatments did not record differences in TVFA's, ammonia concentration and pH value of rumen liquor at 0, 3 and 6 h post feeding. Also no significant differences were observed among the experimental groups in blood metabolites, while albumin, cholesterol and progesterone concentration increased ($p<0.05$) as animal age increased. Groups fed TRSV and TRSR recorded lower ($p>0.05$) values in total gain, average daily gain and worst ($p>0.05$) feed conversion as DM and CP compared to the control group. It could be concluded that using biologically treated rice straw in ewe-lambs ration had no adverse effects on nutrients digestibility and nitrogen balance as well as physiological response, while insignificantly decreased ($p>0.05$) growth performance.

Key words: Biological treatment, digestibility, nitrogen balance, physiological response

INTRODUCTION
The economic return of a sheep production depends on many factors, including animals genetic potentiality, management and level of nutrition and the costs of the major inputs such as feed. For replacement breeding females, the age at puberty is highly affect the ewe's overall lifetime productivity and the economic return above the rearing cost. At tropical area, photoperiod is not an important factor in reproductive cycle of sheep (Dyrmundsson, 1973). This makes the level of feeding and management is the main important influence on onset of puberty (Johnson *et al*., 1988). Due to high shortage of conventional fodders especially in arid and semi-arid areas such as Egypt, the big feed gap between the requirements and the available sources necessitates great efforts to realize the best utilization of the available feed resources. During the last years, many efforts have been employed to increase their feeding values by biological treatments (Liu and Orskov, 2000; Zhu *et al*., 2005; Eun *et al*., 2006), which many studies indicated that the biological treatments tend

to increase *in vitro* digestibility of tested materials (Zadrazil and Puniya, 1995; Sharma and Arora, 2010) and *in vivo* digestibility (Chen *et al.,* 1995; Akinfemi *et al.,* 2009; Arora and Sharma, 2009). Moreover, Fazaeli *et al.* (2004), Kabirifard *et al.* (2007), Omer *et al.* (2012), Shrivastava *et al.* (2012) and El-Bordeny *et al.* (2015) reported a positive response in terms of nutrient utilization, nitrogen balance as well as body weight gain associated with using of biologically treated crop residues in animal feeding. However, little researches have been done to evaluate effect of inclusion biologically treated crop residues on animal physiological performance. So, the objective of this study was to investigate effect of inclusion biologically treated rice straw with *Trichoderma viride* or *Trichoderma reesei* in growing ewe-lambs ration on their productive and physiological response.

MATERIALS AND METHODS

This study was conducted at Mahallet Mussa, farm station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and laboratories of Animal Nutrition, Department of Animal Production, Faculty of Agriculture, Ain Shams University, Egypt. Microorganisms and Biological treatment: *Trichoderma reesei* and *Trichoderma viride* were obtained from Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Minufiya University. The biological treatments were applied according to El-Bordeny *et al.* (2015). Then dried in dry and sunny place for 7 days and stored until used in animal rations.

Growth trial: Thirty crossbred (Rahmany× Romanof) ewe-lambs (three months old and 17±1.13 kg body weight) were randomly allocated to three groups, 10 ewe-lambs each. Each group was assigned randomly to receive one of the three experimental rations. The experimental rations were formulated to cover ewe-lambs allowances according to NRC (1985). The first ration (control) contained 55% concentrate feed mixture and 22.5% clover hay plus 22.5% Untreated Rice Straw (URS), while treated rice straw with *T. reesei* (TRSR) or *T. viride* (TRSV) were replaced by URS in the second and third rations, respectively. Formulation and chemical composition of the experimental rations are presented in Table 1. Complete rations and fresh water were offered twice

Table 1: Chemical composition of ration ingredients and experimental rations

Items	Ingredients					Ration		
	URS	TRSv	TRSr	Hay	CFM	T1	T2	T3
Constituents (g kg^{-1} DM)								
Organic matter	822.4	804.8	807.5	891.9	875.0	867.0	863.0	863.6
Ash	177.6	195.2	192.5	108.1	125.0	133.0	137.0	136.4
Crude protein	53.0	101.6	68.1	130.5	161.0	129.8	140.8	133.2
Crude fiber	421.3	386.8	378.4	355.1	140.1	251.8	244.0	242.1
Ether extract	16.6	19.5	18.5	19.4	42.9	31.7	32.2	321.2
Nitrogen free extract	331.5	296.9	342.5	386.9	531	453.7	445.9	456.2
Cell wall constitutes (g kg^{-1} DM)								
Neutral-detergent fiber	687.4	634.5	651.9	740	390	535.7	523.8	527.7
Acid-detergent fiber	508.3	497.0	478.8	555	230	365.7	363.2	359.1
Acid-detergent lignin	83.7	105.3	104.5	87.0	630	384.9	389.8	389.6
Hemicellulose	179.1	137.5	173.1	468	167	237.5	228.1	236.1
Cellulose	424.6	391.7	374.3	185	160	225.2	217.8	213.8
Calculated feeding values								
Calculated GE (kcal)	3373	3317	3322	3993	4090	3907	3894	3895
Calculated DE (kcal)	2563	2521	2525	3034	3108	2969	2959	2960
Calculated TDN (%)	58.14	57.17	57.27	68.81	70.50	67.34	67.12	67.14

daily at 8.00 and 17.00 in quantities sufficient to allow free choice access to the ration. The growth phase lasted 180 days, the animals were weighed every two week to calculate total and daily gain and feed conversion.

Digestion and nitrogen balance trials: Twelve mature crossbred (Rahmany×Roman of) rams (three years old and 60 kg live weight) were randomly assigned to three groups, four animals each to evaluate the experimental rations through metabolic trials (21 days for adaptation and 7 days for sample collection). Each group was assigned randomly to receive one of the three experimental rations in amount sufficient to cover its maintenance requirements. Feces samples were collected daily and dried at 60-70°C in a hot air oven. Volume of 50 mL diluted sulfuric acid (10%) was butted in urine collect containers each day. A representative samples (10%) of urine volume were stored for nitrogen determination. The orts were weighted daily, meanwhile feed intake was calculated. The dried samp1es of feces and feeds were grinded to pass through 1 mm screen, then, these samples of rations and feces were stored for chemical analysis. Consequently, the nutrients digestibility and nutritive values of the experimental rations were calculated as well as nitrogen balance.

Rumen parameters: Rumen liquor samples were taken just before morning feeding, three and six hours post feeding from 4 ewe-lambs of each group. Samples of rumen liquor were strained through four layers of cheese cloth and its pH was immediately measured after collection using pH meter (HANNA, Italy). Strained rumen liquor samples were acidified with 0.1 M hydrochloric acid and concentrate orthophosphoric acid and stored by freezing for determination of Total Volatile Fatty Acids (TVFA's). Ammonia was separated from rumen liquor by steam distillation, collected in boric acid solution and determined by titration with standard acid (Preston, 1995). Total VFA was determined by steam distillation according to Cunniff (1997).

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

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

Blood parameters: At 60, 120 and 180 days of the experiment, blood samples were taken from all experimental animals. A sample of 10 mL of blood per animal was withdrawn from the jugular vein. The blood sample was directly collected into a clean dried glass culture tubes (after addition of heparin as an anti coagulant) at 3 h post feeding. The blood plasma was obtained by centrifuging the blood samples soon after collection at 4000 (rpm) for 15 min. Blood plasma was transferred into a clean dried glass vials and then stored in deep freezer at -20°C for subsequent specific chemical analysis. Blood plasma samples constitutes were determined using commercial kits (Boi-diagnostics Company, Egypt). Direct radioimmunoassay technique (RIA) was used for plasma progesterone determination using ready antibody coated tubes kit (Immunotech, A Beckman Coulter Company, France).

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

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

where, y_{ij} is represents observation, μ is overall mean, T_i is effect of treatment (experimental group), e_{ij} is experimental error. While, the model used for statistical analysis of rumen liquor and blood metabolites was:

$$Y_{ij} = \mu+T_i +S_j+an\ (t)+S*T+e_{ijk}$$

where, Y_{ijk} is the observation on the ith treatment jth time, μ is overall mean, T_i is Effect of the ith treatment, S_j is Effect of the j th time, an (t) is Effect of the animal in the treatment and e_{ijk} is Random experimental error.

RESULTS AND DISCUSSION

Nutrients digestibility and nitrogen efficiency utilization: Insignificant differences were observed among the different experimental groups in DM, OM, CF, CP, EE and NFE digestibility and feeding values as digestible protein and TDN (Table 2). These may be due to that the biologically treated rice straw had lower calculated feeding values as Gross Energy (GE), Digestible Energy (DE) and Total Digestible Nutrients (TDN) (Table 1) which the fungi utilize the soluble carbohydrate, then, the structure carbohydrate contents as carbon source to produce energy and CO_2, to grow up and produce its biomass consequently decrease the easy fermented substrates (El-Bordeny *et al*., 2015) as well as the lower proportion of untreated and biologically treated rice straw in ration formula which was represent about 22.5% of the total ration. Similar trend were observed by El-Bordeny *et al.* (2015) which insignificant differences among the different experimental group in nutrient digestibility due to inclusion biologically treated rice straw at rate

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

Items	T1	T2	T3	SEM	p-value
Nutrient digestibility					
DM	66.81	67.27	66.49	1.31	0.76
OM	69.12	69.93	68.68	1.18	0.75
CF	50.30	50.50	49.60	1.24	0.86
EE	75.10	77.40	74.50	2.03	0.60
CP	61.11	61.71	59.18	2.27	0.72
NFE	78.72	79.11	80.36	1.09	0.08
Rations feeding values (%)					
DCP	7.93	8.69	7.88	0.31	0.10
TDN	68.72	70.85	71.26	1.06	0.27
Nitrogen efficiency utilization (g day^{-1})					
Nitrogen intake	16.68	18.70	17.31		
Fecal nitrogen	6.96	7.63	7.69	0.59	0.63
Digested nitrogen	9.73	11.07	9.61	0.59	0.23
Urinary nitrogen	5.06	4.95	3.96	0.47	0.27
Nitrogen retention	4.67	6.11	5.65	0.64	0.33
Nitrogen efficiency utilization	28.00	32.70	32.70	3.67	0.61

Values in this table revealed the data collected from the digestion trails are significantly ($p<0.05$) different

24%. While, many researches reported that, the biological treatments of crop residues tend to increase nutrients digestibility (Chen *et al*., 1995; Akinfemi *et al*., 2009; Arora and Sharma, 2009). This variability in responses to the biologically treated materials feeding is due to that different condition, such as ration formula, using another source of roughage material and level of concentrate roughage ratio (El-Bordeny *et al.,* 2015).

While, insignificant increase in nitrogen retention and nitrogen utilization efficiency were observed for the groups received rations containing treated rice straw (T2 and T3) compared to control group (Table 2). These could be understood through the higher CP content recorded for the rations contained TRSV or TRSR (Table 1) parallel to the insignificant differences in nutrients digestibility. These results agreed with the findings obtained by El-Bordeny *et al*. (2015), who observed insignificant differences among the different groups in nitrogen retention. On the contrary, Kabirifard *et al.* (2007), Omer *et al.* (2012), Shrivastava *et al.* (2012) and Mahesh and Mohini (2013) found that using biological treated crop residues in animal feeding improve nitrogen efficiency of utilization.

Rumen liquor parameters: Insignificant differences were observed among the different experimental groups in rumen liquor TVFA's, ammonia concentration and pH values at 0, 3 and 6 h post feeding (Table 3). These may be attributed to that the three experimental rations were approximately similar in chemical composition (Table 1) as well as the insignificant differences recorded in nutrient digestibility (Table 2). Total VFA's concentration in the rumen depends on factors, such as animal diet, absorption rate, sampling time and activity and count of rumen microbial population (McDonald *et al*., 1995). While, significant decrease in rumen liquor total nitrogen and non protein nitrogen (mg dL^{-1}) for T2 at ($p = 0.06$) compared to T1 and T3 (Table 3). The present results agree with the findings obtained by El-Bordeny *et al.* (2015), who reported insignificant differences in rumen fluid TVFA's concentration among the animals received ration containing biological treated and untreated rice straw. However, Akinfemi and Ladipo (2011) Omer *et al.* (2012) and Mahesh and Mohini (2013) reported that inclusion biologically treated crop residues in animal rations led to increased concentration of rumen liquor TVFA's and ammonia compared control.

Growth performance: Data in Table 4 showed that, total gain and average daily gain were insignificantly decreased for groups fed ration contained biological treated rice straw compared to control group. Also feed conversion as DM, CP and TDN were insignificantly ($p \leq 0.05$) deteriorated for groups fed ration contained treated rice straw compared to control group. The insignificant reduction recorded for groups G2 and G3 could be attributed to the recorded insignificant differences in intake of DM, CP and TDN as well as in nutrient digestibility parallel to the insignificant decrease in average daily gain. The present findings are disagree with the findings

Table 3: Effect of treatments on rumen liquor parameters

	Zero time			After 3 (h)			After 6 (h)			p-value			
Items	T1	T2	T3	T1	T2	T3	T1	T2	T3	SE	Trt	Tim	Trt*Tim
TVFA's (meq dL^{-1})	6.88	6.61	6.63	13.51	12.84	13.26	8.88	8.96	7.77	0.48	0.92	0.001	0.91
NH_3-N values (mg dL^{-1})	19.83	25.43	24.03	35.47	34.13	35.23	35.70	28.88	31.27	1.25	0.95	0.001	0.10
pH values	7.03	6.83	6.85	5.80	6.03	5.75	6.18	6.50	6.33	0.56	0.23	0.001	0.10
Total nitrogen (mg dL^{-1})	245.00	183.80	227.50	205.60	183.80	323.80	529.40	455.00	490.00	17.28	0.06	0.001	0.66
Non protein nitrogen (mg dL^{-1})	99.20	70.00	110.80	221.70	198.30	233.30	280.00	367.50	309.20	15.39	0.71	0.001	0.17
True protein nitrogen (mg dL^{-1})	145.80	113.80	116.70	58.90	43.80	99.20	249.40	87.50	180.80	24.94	0.10	0.08	0.37

Table 4: Effect of treatments on lambs growth performance and economic efficiency

Items	T1	T2	T3	SEM	p-value
Body weight (kg)					
Initial weight	17.00	17.00	17.00	1.46	0.99
Final weight	40.85	39.43	40.00	1.61	0.98
Total weight gain (kg)	23.85	22.43	23.00	1.06	0.97
Average daily gain (kg)	0.133	0.125	0.128	0.008	0.97
Feed intake (kg h^{-1} day^{-1})					
Dry matter intake	918.30	916.40	917.10		
Crude protein intake	119.20	125.60	124.80		
Total digestible nutrients intake	631.10	649.30	653.50		
Feed conversion (kg kg $gain^{-1}$)					
Dry matter conversion	8.20	8.70	8.49		
Crude protein conversion	1064.00	1225.00	1131.00		
Total digestible nutrients conversion	5.64	6.16	6.05		

Table 5: Effect of treatments on some blood plasma parameters

	At 60 (days)			At 120 (days)			At 180 (days)				p-value		
Items	T1	T2	T3	T1	T2	T3	T1	T2	T3	SE	Trt	Tim	Trt*Tim
Total proteins (g dL^{-1})	6.90	7.310	7.520	6.900	8.380	6.190	8.760	7.100	9.400	0.97	0.454	0.085	0.563
Albumin (g dL^{-1})	3.14	2.980	3.570	3.330	3.850	3.280	4.340	4.080	4.720	0.67	0.822	0.024	0.705
Globulin (g dL^{-1})	3.76	4.330	3.950	3.570	4.530	2.910	4.420	3.020	4.680	0.32	0.179	0.410	0.324
Urea (mg dL^{-1})	33.70	37.970	32.050	40.150	43.370	51.980	41.500	33.410	32.190	6.71	0.599	0.254	0.274
Creatinine (mg dL^{-1})	0.76	0.720	0.560	0.720	0.750	0.740	0.850	0.730	0.820	0.11	0.067	0.512	0.251
Cholesterol (mg dL^{-1})	34.48	36.090	33.730	31.510	33.340	33.730	69.820	62.280	66.380	8.66	0.880	0.002	0.462
Triglyceride (mg dL^{-1})	23.13	31.130	20.030	24.530	37.110	19.830	34.210	28.180	30.040	16.30	0.396	0.484	0.381
Progesterone (g dL^{-1})	0.153	0.100	0.056	0.365	0.596	0.363	0.587	0.603	0.438	0.16	0.88	0.05	0.98
ALT (U L^{-1})	27.35	27.950	30.270	22.880	27.010	24.670	24.020	25.280	33.270	3.80	0.263	0.743	0.400
AST (U L^{-1})	65.00	70.880	72.670	70.330	63.710	61.670	78.670	63.040	56.670	2.27	0.017	0.030	0.06

of Fazaeli *et al.* (2004), Kabirifard *et al.* (2007), Khattab *et al.* (2009), Omer *et al.* (2012) and Shrivastava *et al.* (2012), who reported a positive response in terms of nutrient utilization, nitrogen balance as well as body weight gain associated with utilization of biological treated crop residues in animal rations.

Blood parameters and physiological response: Data of Table 5 showed that inclusion biological treated rice straw in female lams ration resulted in insignificant (p>0.05) differences in total plasma protein, albumin, globulin and urea concentration at 60, 120 and 180 days of the experiment. These may be due to that the three experimental rations were balanced in their chemical composition, specially crude protein (Table 1) which the protein contain in animal diets is the main limiting factor that affect blood proteins concentration and as reported in other studies (Chumpawadee *et al*., 2006; Javaid *et al*., 2008), blood urea concentration correlated to ruminal ammonia concentration. Thus, the present results clearly indicate that protein metabolism is mostly unaffected by inclusion biologically treated rice straw in ewe-lambs rations as well as the level of dietary protein is satisfactory to cover animal allowances. Serum total protein and albumin reflect the nutritional status of the animal and it has a positive correlation with dietary protein and the low level of plasma proteins may be attributed to a decrease in the protein synthesized and absorbed and an increase in protein losses (Bush, 1991). Similar results were obtained by Oh *et al.* (2010) and El-Bordeny *et al.* (2015), when fed rations contained biologically treated rice straw.

Insignificant (p>0.05) differences among the different experimental groups in plasma creatinine concentration at 60, 120 and 180 days were observed (Table 5). From the results of plasma urea and creatinine concentrations, it is clear that experimental animals were not in a catabolism situation and kidney function was not adversely affected by biological treatments and the animals were in a good nutritional condition (El-Bordeny *et al*., 2015). Generally, serum creatinine is an indicator of glomerular filtration in the kidney.

Moreover cholesterol, triglyceride and progesterone concentration were not significantly affected by inclusion biologically treated rice straw at 60, 120 and 180 days of the experiment (Table 5). Also the values of AST and ALT presented in Table 4 showed normal hepatic tissues activity of the animal consequently; inclusion of biological treated straws in ewe-lambs rations could be used without any adverse effect on the liver functions.

The data of Fig. 1 showed that no significant differences were observed among the growth patterns of the animal in different experimental groups, which indicated that inclusion of the biological treated rice straw in ewe-lambs ration had not any adverse effect on animal growth pattern, which level of feeding is the main important factor that affect onset of puberty (Johnson *et al*., 1988) It is clear from data of Table 5 that albumin, cholesterol and progesterone concentration increased gradually along the experimental period as the animals grow towards puberty as well as insignificant differences were observed among the different experimental groups in albumin, cholesterol and progesterone concentration, which mean that using biologically treated rice straw in ewe-lambs ration had not bad effect on sexual growth and age of puberty of the experimental animals. The elevated level of progesterone in the different experimental animal is highly indicator on onset of puberty. Circulating cholesterol concentration is known to be affected by thyroid function (Laird, 1972) and the activity of steroid producing organ (Roussel *et al*., 1982), therefore, the not significant variations in cholesterol and progesterone concentration among the different treatments indicated that the activity of thyroid and steroids organs was not adversely affected for all groups. Thus, ewe-lambs that have received biologically treated rice straw could have the same physiological response for those received control ration. In addition, increased cholesterol levels may lead to greater reproductive hormone synthesis. It is obvious from data of Fig. 1, 2 and 3 that the main factors that affect the animal sexual growth are animal age and body weight with high correlation (r = 0.81) was observed between body weight and progesterone concentration, while the correlation between total blood cholesterol and progesterone concentration was (r = 0.45) in this connection (Bianchi *et al*., 2014) found a higher concentration of circulating cholesterol is positively correlated to progesterone levels (r = 0.74).

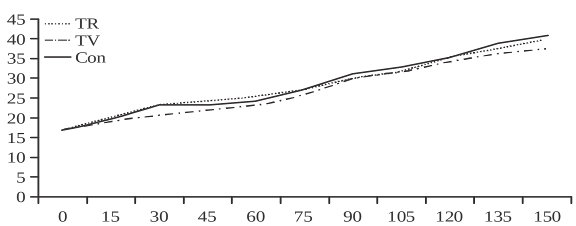

Fig. 1: Effect of biological treatment on body weight along the experimental period

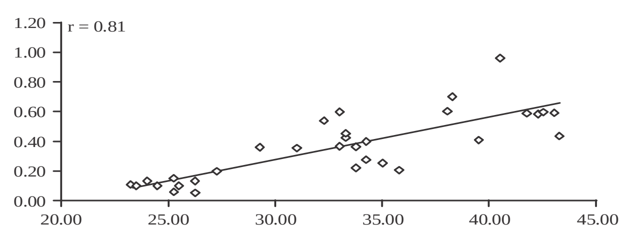

Fig. 2: Linear correlation between body weight and progesterone concentration in female lambs

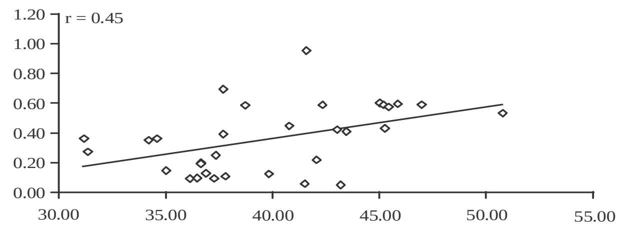

Fig. 3: Linear correlation between total blood cholesterol and progesterone concentration in female lambs

CONCLUSION

Using biologically treated rice straw in ewe-lambs rations had no adverse effects on nutrients digestibility, rumen fermentation and nitrogen balance. Also, using the bio-upgraded rice straw was not affected blood plasma metabolites and progesterone level as well as age of puberty.

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