### Chemical, Biological and Biochemical Treatments to Improve the Nutritive Values of Sugarcane Bagasse (SCB): 1- Chemical Composition, Scanning Electron Microscopy, *In Vitro* Evaluation, Nutrients Digestibility and Nitrogen Utilization of Untreated or Treated SCB

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Abstract: The present study aimed to evaluate the effect of chemical (3% urea), biological (fungi, yeast and bacteria and their combinations) and biochemical treatment (combined biological + urea) on the nutritive value of sugarcane bagasse. The effect of treatments on chemical composition, cell wall constituents, scanning electron microscopy, *in vitro* DM and OM disappearance and *in vivo* nutrients digestibility of bagasse was studied along with N- utilization with lambs. The results showed that different treatments increased DM, CP, EE and ash, while decreased OM, CF and NFE. The increments in CP content were 305, 188 and 156% due to biochemical, chemical and biological treatments, respectively. The chemical, biological and biochemical treatments decreased NDF, ADF, hemicellulose and cellulose, while increased ADL content. The obvious change in the structure of cell parenchyma was observed in chemical, biological and biochemical treated bagasse. Values of *in vitro* dry matter and organic matter disappearance were significantly higher (P<0.01) for biochemical, chemical and biological treated bagasse, respectively, than untreated bagasse. The nutritive values and N-utilization recorded with lambs fed rations containing biological and biochemical treated sugarcane bagasse were greater than those containing untreated or urea treated ones. It was concluded that, different treatments improved chemical composition, cell wall constituents, IVDMD and IVOMD disappearance, almost nutrients digestibility, TDN and DCP values of sugarcane bagasse with the superiority of fungi or fungi + bacteria + urea treatments.

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Key words: Sugarcane bagasse, chemical and biological treatments, chemical composition, *in vitro*, scanning electron microscopy, digestibility.

### 1. Introduction

Shortage in the animal feeds is a well-known problem; therefore several studies were carried out to improve the nutritive values of the poor quality roughages to find out an effective and practical solution to overcome the feed shortage problem in animal feeds and resources.

The estimated amount of poor quality roughages in Egypt includes rice straw, wheat straw, bean straw, corn stalks, corn cobs, rice hulls and sugarcane bagasse is about 19 million tons/ year (El-Shinnawy and Shoukry, 2002). Among these roughages, sugarcane bagasse represents about 4.13 million tons/ year according to Agriculture Economic and Statistics Institute (1995). Under this circumstance, sugarcane becomes important as a potential feed resource given its high biomass yield and adaptation to the environment (Conrad *et al.*, 1990 and Molina, 1990).

Sugarcane bagasse, filter cake and pith are secondary by-products in the process of sugar production. The main components of them are cellulose, hemicellulose and lignin. As a result of its high content of lignin, ruminal digestion is inhibited and thus, the nutritive value of bagasse and pith is low for ruminants.

To improve the nutritive value of these agriculture residues, it is important to breakdown the linkages among cellulose and lignin by mechanical, chemical or biological and combined biological plus chemical treatments. Many scientists suggested the use of ammonia and urea to increase the crude protein contents of the poor quality roughages (Shoukry *et al.*, 1992 & Fouad *et al.*, 1998).

Physical and chemical pre-treatment such as ammonia explosion in combination with fungal may also upgrade the nutritional quality of marginal agricultural residues such as bagasse.

Biological treatment is an alternative method to modify the fibrous materials by ruminants. The mode of fungal decay on roughages using the white rot fungi was shown to improve *in vitro* dry matter digestibility of the decayed substrate (**Dawson** *et al.*,1990). Recently the production of microbial protein from agricultural crop residues received that attention of several workers (El-Ashry *et al.*, 1997 and 2002; Deraz and Ismail, 2001 and Kholif *et al.*, 2001). The objective of this work was to study the possibility of improving utilization of sugarcane bagasse as feeds for ruminants using chemical, biological and biochemical treatments. The effect of different treatments on chemical composition, cell wall structure, IVDMD and IVOMD of sugarcane bagasse was studied. The effect of the best treatments detected on nutrients digestibility and N balance with lambs was also investigated.

### 2. Materials and Methods

This study was carried out at the Department of Animal Production, National Research Center, Dokki, Giza, Egypt and the Experimental Farm Station belongs to Faculty of Agriculture, Animal Production Department, Al-Azhar University, Nasr City, Cairo, Egypt.

### Sugarcane by-product preparation:

Bagasse type of low quality roughages was obtained from Sugar Company in Edfo City, Aswan, Egypt, was sun dried to 90% DM and chopped to an approximate 1-3cm.

### **Chemical treatment:**

The required amount of urea (30g) was dissolved in 500 ml water and sprayed on 1kg of chopped sugarcane bagasse. The treated bagasse were thoroughly to be homogenous then ensiled up to 3 weeks.

### **Biological treatment:**

Fungi (*Phanerochaete chrysosporium* NRRL-6361), bacteria (*Cellulomonas uda* NRRL-404) and yeast (*Candida utilis* NRRL-1084) were obtained from the Genetic and Cytology Department, National Research Center, Dokki, Giza, Egypt.

The microorganisms were maintained on agar medium composed of (g/l) yeast extract, 3.0; malt extract, 30; peptone, 5.0; sucrose, 20 and agar, 20.

### **Biochemical treatment:**

At the end of fermented period of biological sugarcane bagasse, it aerate overnight then mixed well with urea then bagged and stored up to 15 days. This study included two parts of experiments: The first part (laboratory trials):

The first part was laboratory trials which were carried out to study the effect of using chemical treatment (3% urea), biological treatment (fungi,

yeast, bacteria and combined fungi plus yeast; fungi plus bacteria and bacteria plus yeast) and biochemical treatment (biological treatment followed by chemical treatment) on chemical composition, cell wall constituents and scanning electron microscopy of sugarcane bagasse.

Treatments were designed as follow:

- T<sub>1</sub>: Bagasse untreated (Unt.)
- T<sub>2</sub>: Bagasse treated with 3% urea (urea Tr.)
- T<sub>3</sub>: Bagasse treated with fungi (F Tr.)
- T<sub>4</sub>: Bagasse treated with bacteria (B Tr.)
- T<sub>5</sub>: Bagasse treated with yeast (Y Tr.)
- T<sub>6</sub>: Bagasse treated with fungi + yeast (F+Y Tr.)
- T<sub>7</sub>: Bagasse treated with fungi + bacteria (F+B Tr.)
- T<sub>8</sub>: Bagasse treated with bacteria + yeast (B + Y Tr.)
- T<sub>9</sub>: Bagasse treated with fungi + urea (F + urea Tr.)
- $T_{10}$ : Bagasse treated with bacteria + urea (B + urea Tr.)
- $T_{11}$ : Bagasse treated with yeast + urea (Y + urea Tr.)
- T<sub>12</sub>: Bagasse treated with fungi + yeast + urea (F+ Y +urea Tr.)
- $T_{13}$ : Bagasse treated with fungi + bacteria + urea (F+B+ urea Tr.)
- $T_{14}$ : Bagasse treated with bacteria + yeast + urea (B+ Y+ urea Tr.)

### The second part (in vitro and in vivo trials):

The second part was carried out to evaluate *in vitro* dry matter and organic matter disappearance for bagasse with different chemical, biological and biochemical treatments under study. The treatments gave the best results were *in vivo* evaluated by digestibility and N-balance trials with lambs.

### **Digestibility and N-balance trials:**

Twenty Ossimi male lambs with an average live body weight 32.7 kg and 180 days age were randomly assigned into five nutritional treatments (each of 4 animals) to receive one of the following roughages:  $R_1$ , berseem hay;  $R_2$ , untreated sugarcane bagasse;  $R_3$ , bagasse treated with 3% urea;  $R_4$ , bagasse treated with fungi and  $R_5$ , bagasse treated with fungi + bacteria + 3% urea. The composition of the five experimental rations is presented in Table (1). Chemical composition and cell wall constituents of different experimental rations are presented in Table (2).

Rations were offered to lambs *ad lib*, while, water and salt blocks were freely available all day time.

Table	(1):	Com	position	of	experimental	rations
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Itom	Experimental rations, %						
Item	R <sub>1</sub>	$\mathbf{R}_2$	R <sub>3</sub>	<b>R</b> <sub>4</sub>	<b>R</b> <sub>5</sub>		

- Berseem hay	30	-	-	-	-
- Untreated bagasse (Unt.B.)	-	30	-	-	-
- Bagasse treated with 3% urea (Urea Tr. B.)	-	-	30	-	-
- Bagasse treated with fungi (F Tr. B.)	-	-	-	30	-
-Bagasse treated with fungi + bacteria + 3% urea (F +B					
+urea Tr. B.)	-	-	-	-	30
- Ground corn grain	51	51	51	51	51
- Sunflower meal	7	7	7	7	7
- Wheat bran	7	7	7	7	7
-Limestone, sodium chloride and minerals & vit. mix.	5	5	5	5	5
Total	100	100	100	100	100

Animals were individually confined to wooden metabolic crates. Digestibility and N-balance trials were carried out to determine nutrients digestibility, nutritive values and N-balance for the five experimental rations. Digestibility trials consisted of 21 days, where 14 days were considered as a preliminary period to allow animals a suitable adaptation followed by 7 days for total collection of feces and urine. Composite samples from collected feces and urine of each animal were taken for chemical analyses. Samples of rations offered and residuals if any, were weighed daily during the collection period for further chemical analysis.

Table (2): Chemical composition and cell wall constituents of the experimental rations (on DM basis, %)

Itom	Experimental rations									
110111	<b>R</b> <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	<b>R</b> <sub>4</sub>	R <sub>5</sub>					
DM	91.79	91.48	91.18	90.70	90.73					
OM	93.41	96.35	95.57	95.20	96.65					
СР	12.09	8.91	9.93	9.75	10.32					
CF	18.24	23.86	21.83	21.66	21.55					
EE	3.69	3.23	3.27	3.27	3.91					
NFE	59.39	60.35	60.54	60.52	60.87					
Ash	6.59	3.65	4.43	4.80	3.35					
NDF	42.76	38.34	41.65	42.93	40.22					
ADF	19.14	16.85	25.49	25.50	26.30					
ADL	5.99	2.68	7.76	8.76	9.12					
Hemicellulose*	23.62	21.49	16.16	17.43	13.92					
Cellulose**	13.15	14.17	17.73	16.74	17.18					

\* Hemicellulose = NDF - ADF

The Proximate chemical analysis of untreated and treated bagasse, berseem hay, concentrate feed mixture (CFM), feces and urine was determined according to A.O.A.C. (1990). Nitrogen free extract (NFE) was calculated by difference. Cell wall constituents were analyzed according to Goering and Van Soest (1970), to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose and cellulose were determined by difference. The electronic microscope scanning for untreated and treated bagasse was done according to Baker *et al.* (1986) using Electron Probe Microanalyzer.

#### In vitro evaluation:

The *in vitro* dry matter and organic matter disappearance (IVDMD and IVOMD) were determined according to the method described by **Tilley and Terry (1963).** This was done on triplicate samples; rumen liquor was collected from cannulated Rahmani sheep using a stomach tube. The rams were \*\* Cellulose = ADF - ADL

maintained on all berseem hay diet for a period of at least 3 weeks before collecting the liquor. Hay was offered to the animals at a feeding level of 120% maintenance.

### Statistical analysis:

Data concerning *in vitro* DM and OM disappearance and *in vivo* nutrients digestibility and N-balance trials were statistically analyzed according to **SAS (1998)**. A one-way classification analysis followed by Duncan's multiple-range test (**Duncan**, **1955**) for testing the significance between means was used.

### 3. Results and Discussion Chemical composition:

Results of chemical composition of untreated and treated bagasse with different treatments are presented in Table (3). The data obtained showed that DM content was increased after treatment in different cases, the highest DM content was observed in bagasse treated with 3% urea (96.2%). On the contrary, the lowest value was recorded in untreated bagasse (91.9%). On the other hand, different biochemical treatments showed higher values in comparison with the biological treatments. Higher OM values were recorder with T<sub>5</sub> (96.6%). On the contrast the lowest value (82.4%) was recorded in T<sub>14</sub> as a biochemical treatment, while untreated and treated bagasse with urea indicated intermediate values (94.7 and 92.3%). These results agree with those obtained by Chandra et al. (1991) who found that the decreased OM was a reflection to decrease in CF which was utilized by fungi, while the increase in total ash was inversely related to the OM content of treated paddy straw (untreated, T. viride, A. niger and mixed fungi treated paddy straw).

On the other hand, the increments in CP content (304, 188 and 156%) were due to biochemical, chemical and biological treatments, respectively. These results agree with those obtained by several authors (Talha, 1990; Tabana, 1994; Mohamed, 1998 and Mousa et al., 1998). These effects were mainly due to nitrogen content of added urea in the chemical treatments, microbial protein from biological treatment due to growing fungi and bacteria and also to the biochemical treatments. Chandra et al. (1991) found that the increase in CP was reflected by a decrease in CF content, while, Yang et al. (2001) showed that solid state fermentation changed the composition of the straw. The protein content increased from 6.7% in unfermented straw to 14.7% in fermented straw.

Generally, biochemical treatment was the best treatment which led to decrease CF content followed by T<sub>7</sub> as a biological treatment and 3% urea as a chemical one. The decline in crude fiber content of the experimental rations could be resultant of the enzymes secreted by the biological treatment (Gado *et al.*, 2007). The decrease in CF content by urea treatment such as sodium hydroxide treatment may be due to the liberation of cellulose from its bonds with lignin (delignification) which increased the solubility (Abd El-Ghani *et al.*, 1999).

As shown in Table (3) different biochemical treatments led to increase EE contents. These increase in EE due to synthesis of fatty acids through growth of bacteria (Gado *et al.*, 2007). Zadrazil *et al.* (1995); Neclakantan and Singh (1998) and Rane and Singh (2001) who found that the products of solid state fermentation gets in enriched fats, soluble sugars, vitamins and amino acids and can be used entirely as in animal feed.

There were decreases in the NFE contents of bagasse from 42.24% in untreated to 42.10, 38.21 and 37.56% in chemical, biological and biochemical treated bagasse, except  $T_5$  and  $T_6$  which were increased (44.7 and 43.78%), respectively, whereas the lowest value (32.57%) was recorded in  $T_{14}$ . On the other side, all biochemical treatments indicated lower values compared with the untreated bagasse.

Generally, all biochemical and chemical treatments increased ash content plus some biological treatments, compared with the untreated bagasse. These effects were due to added media of growing bacteria, fungi and yeast then degradation of DM to ash and OM. Chandra et al. (1991) found that the increased total ash on all treatments were a reflection to the decrease in CF and NFE contents. It could be concluded that all treatments had great effects on degradation of CF from 49.5 to 39.10% and increasing CP content from 1.8 to 8.8% of treated bagasse. The present finding is in agreement with Bakshi and Langer (1991), who reported that CF decreased from 42.92 to 17.87% in the compost and spent in a treatment with cellulose enzymes. Supportive results were reported by Streeter et al. (1982); Reader and Mc Queen (1983); Eduardo and Etienne (1985) and Lawrance and Abada (1987). Abd El-Galil (2000) who found that, when bagasse treated by Cellulomonas sp. bacteria CF was decreased from 44.9 to 30.21% and CP was increased from 1.75 to 15.9% with a reduction in DM content.

The present results confirmed the results obtained by **Shoukry** *et al.* (1985) and Abdul-Aziz *et al.* (1997). Gado (1999) fermented rice straw and bagasse with *Trichoderma ressei* and reported that CF, EE, NDF, ADF, cellulose and hemicellulose contents were lowered significantly (P<0.05) in both treated rice straw and bagasse. Badr (2001) found that biological treatment by *P. florida* decreased CF content than that in raw material, being 3.5%, while combined fungi and bacteria at level of 3.0% were more effective in decreasing CF content from 37.85% to 18.42%, followed by incubation of corn stalks by *P. florida* and *E. carotovora* at level 2% (being 47.37% CF for control).

Shoukry *et al.* (1985) found an increase in CP, EE and ash content when treated sugarcane bagasse with four different microorganisms. **Deraz and Ismail (2001) and El-Ashry** *et al.* (2001) reported that fungal treatment led to decrease OM and CF contents, while, CP and ash contents increased compared with the untreated roughages.

Table (3): Effect of chemical, biological and biochemical treatments on chemical composition of sugarcane bagasse (on DM basis,%)

Item	DM	OM	СР	CF	EE	NFE	Ash

T <sub>1</sub> :Unt.	91.90	94.70	1.80	49.50	1.16	42.24	5.30
Chemical treatment :							
$T_2$ :Urea Tr.	96.20	92.30	5.20	43.70	1.30	42.10	7.70
Biological treatments:							
$T_3$ :F Tr.	94.10	90.20	4.80	45.80	1.58	38.02	9.80
T <sub>4:</sub> B Tr.	94.10	87.90	4.30	45.60	1.44	36.56	12.10
$T_{5}$ Y Tr.	93.90	96.60	4.80	45.80	1.30	44.70	3.40
$T_6:F + Y Tr.$	93.95	95.40	4.50	46.70	0.42	43.78	4.60
$T_7:F + B Tr.$	93.80	90.65	4.60	43.20	1.26	41.59	9.35
$T_{8}B + Y Tr.$	93.80	91.30	4.70	48.60	1.33	36.67	8.70
Overall mean				1		1	
of biological treatments	93.94	92.00	4.61	45.95	1.22	40.22	7.99
Biochemical treatments:							
$T_9:F + urea Tr.$	95.37	87.80	8.80	39.94	2.85	36.21	12.20
$T_{10}B + urea Tr.$	94.60	94.10	8.20	42.30	1.96	41.64	5.90
$T_{11:}$ Y + urea Tr.	93.90	87.60	6.20	40.80	2.55	38.05	12.40
$T_{12}F + Y + urea Tr.$	95.92	88.10	7.20	39.10	3.16	38.64	11.90
$T_{13}F + B + urea Tr.$	96.10	91.10	6.50	42.90	3.43	38.27	8.90
$T_{14:}B + Y + urea Tr.$	95.97	82.40	6.77	39.80	3.23	32.60	17.60
Overall mean of biochemical treatments	95.31	88.52	7.28	40.81	2.86	37.57	11.48

### Effect of chemical, biological and biochemical treatments on cell wall constituents:

It is clear from Table (4) that chemical, biological and biochemical treatments had a significant effect on cell wall constituents (CWC) of bagasse, different treatments decreased NDF contents. The highest decrease in NDF content was recorded with bagasse treated by 3% urea. There were slight differences among treatments detected in ADF content. The highest decrease in ADF content was found in bacteria + yeast + urea treated bagasse. Hemicellulose content was decreased in different bagasse treatments except in  $T_{14}$ , while the highest decrease was recorded with urea treated bagasse (20.57%).

Table (4): Effect of chemical,	biological and	biochemical	treatments	on cell	wall (	constituents	of sı	ugarcane
hagasse (%)								0

bagasse (70)					
Item	NDF	ADF	ADL	Hemicellulose.*	Cellulose**
T <sub>1</sub> :Unt.	87.95	58.87	9.97	29.08	48.90
Chemical treatment :					
$T_{2:}$ Urea Tr.	75.81	55.24	13.20	20.57	42.04
Biological treatments:					
T <sub>3</sub> :F Tr.	79.19	57.06	9.53	22.13	47.53
T <sub>4:</sub> B Tr.	81.20	53.50	9.84	27.70	43.66
$T_{5:}$ Y Tr.	82.30	56.37	10.34	25.93	46.03
$T_6:F + Y Tr.$	80.79	52.70	10.09	28.09	42.61
$T_7:F + B Tr.$	83.13	54.13	10.15	29.00	43.98
$T_{8}B + Y Tr.$	81.20	55.30	9.18	25.90	46.12
Overall mean					
of biological treatments	81.30	54.84	9.86	26.46	44.99
Biochemical treatments:					
$T_9:F + urea Tr.$	85.20	58.01	11.47	27.19	46.54
$T_{10}B + urea Tr.$	83.50	58.40	10.33	25.10	48.07
$T_{11}$ :Y + urea Tr.	80.13	58.20	11.22	21.93	46.98
$T_{12}$ :F + Y + urea Tr.	79.90	53.09	9.15	26.81	43.94
$T_{13}$ :F + B + urea Tr.	81.75	53.00	11.37	28.75	41.63
$T_{14}$ :B + Y + urea Tr.	82.15	52.60	11.30	29.55	41.30
Overall mean of biochemical treatments	82.11	55.55	10.81	26.56	44.74

\* Hemicellulose = NDF – ADF

\*\* Cellulose = ADF - ADL

It was noticed that the lowest decrease in cellulose content was recorded with fungi + urea treatment, while the highest decrease was recorded with bacteria + yeast + urea as a biochemical treatment. In general, results indicated that treatments were significant effect on CWC of bagasse. These results agreed with those of Van Soest *et al.* (1984) and Mann *et al.* (1988). Hamissa *et al.* (1985)

treated the fermented bagasse (T. viride fungal) with sodium hydroxide or sodium hypochlorite then it was sprayed with urea solution (4%), they also reported that all treatments significantly decreased CF, NDF, ADF, hemicellulose and NFE content of bagasse, while cellulose and ADF contents were nearly similar. Abdel-Aziz and Ismail (2001) found that NDF, ADF and cellulose contents of fungus treated rice straw decreased by 77.67, 48.81 and 39.73%, respectively. Yang et al. (2001) found that solid state fermentation changed the composition of the straw, hemicellulose and cellulose content decrease from 33.1 and 25.8% in unfermented straw to 26.1 and 16.0% in fermented one, respectively. Deraz and Ismail (2001) reported that CF, NDF, ADF, ADL, cellulose and hemicellulose of cotton stalks significantly decrease when fermented with P. ostreotusor white rot fungi. El-Ashry et al. (1997, 2001 and 2002) and Abdul-Aziz et al. (1997) obtained the similar trend

# Effect of chemical, biological and biochemical treatments on scanning electron microscopy of sugarcane bagasse:

The scanning electron micrograph (SEM) can provide information about the microbial community on the biofilter media. The biomass of individual particle can be mapped. From such precision, important factor such as filter media coverage, thickness and activity can be determined for modelling. The SEM after experiment has already been shown by some researchers (Namkoong *et al.*, **2004** and Chmiel *et al.*, **2005**).



200µm X

### Fig.(1): Scanning electron micrographs (untreated bagasse)

A comparison of the scanning electron microscopic images of sugarcane bagasse before and after treatments is shown in Fig. (1, 2, 3 & 4). Compared to the initial sugarcane bagasse, a biofilm on the surface of the sugarcane bagasse was observed clearly after treatments. An even growth of microbial community on the surface of the pore of the bagasse

is clearly visible. This occurred due to the attacks and decomposition of the microorganisms to cell wall constituents. Initially, the degree of acclimatized depends upon the adaptive capacity of the microorganism in the sugarcane bagasse, substrate concentration and its availability and on other necessary environmental conditions.



Fig. (2): Scanning electron micrographs (chemical treated bagasse)

The tissue of the untreated bagasse (Fig.1) did not reveal any degradation and shows a large amount of debris adhering to the surface of the fibre bundles. because they are coated with non-cellulosic material. chemical, biological Treatments with and biochemical treatments led to relatively cleaner surfaces (Fig. 2, 3 &4, respectively) which supports the removal of wax, pectin, lignin and hemicelluloses as supported by chemical analysis. Unfortunately, the resolution of the available SEM was inadequate to detect fine holes caused by fungal hyphae attacking fibre walls on treated fibre surfaces (Daniel and Volc, 2004). Vazquez et al. (1992) used NaOH, Ca (OH)<sub>2</sub>, NH<sub>4</sub>OH and H<sub>2</sub>O<sub>2</sub> to treated bagasse pith, to investigate their action on single cell protein production using a mixed culture of Cellulomonas flavigena and Xanthomonas sp. SEM analysis showed that pith was mainly composed of microfibrils and parenchymatose cell wall. In addition, light microscopy illustreated swelling of vessels in pith pretreated with Ca (OH)<sub>2</sub> and NaOH, which was evident by the increased pore size of vessels in pith. Kyong et al. (2009) mentioned that the SEM of untreated and ammonia-pretreated rice straw showed that pretreatment induced physical changes in the biomass. Bak et al. (2009) indicated that pretreatment with aqueous-ammonia promoted the removal of external fibers. The removal of external fibers, in turn, led to increased surface area, which may have made cellulose more accessible to enzymes.



Fig. (3): Scanning electron micrographs for biological treated bagasse: (a) fungi, (b) bacteria, (c) yeast, (d) fungi + yeast, (e) fungi + bacteria & (f) bacteria + yeast



yeast, (d) fungi + yeast, (e) fungi + bacteria & (f) bacteria + yeast

## Effect of chemical, biological and biochemical treatments on *in vitro* DM and OM disappearance:

The results from Table (5) showed that different treatments improved IVDMD and IVOMD than untreated, from 12.0 to 26.6% for IVDMD and from 18.0 to 33.23% for IVOMD for untreated and treated bagasse, respectively.

Generally, the best IVDMD value was obtained by  $T_{13}$  followed by  $T_{10}$  as a biochemical treatment and  $T_2$  (3% urea) as a chemical treatment, respectively, the same results were obtained with IVOMD that different treatments increased IVOMD compared with untreated bagasse. The best IVOMD values were obtained by bagasse treated with fungi and treated with bacteria as a biological treatment followed by 3% urea as a chemical treatment and treated with fungi + bacteria + urea and bacteria + urea as a biochemical treatment. These results agreed with those obtained by **Shoukry (1992)**, who found that IVDMD of corn cobs and sugarcane bagasse significantly (P<0.05) increased by 3% urea treatment. **Reis** *et al.* (1995) reported that treated rice straw with 5% urea increased IVDMD. Swidan *et al.* (1996) reported that IVDMD of corn cobs improved by 3 and 5% NH<sub>3</sub> treatment. **Surinder and Suman** (1986) reported that *Pleurotus ostreatus* and *S. pubverulentum* used as the biological treatment of paddy straw produce increases in IVDMD. Shah and **Rehman (1988)** noticed that IVDMD increased when cotton seed hulls fermented by *Bacillus polymexa* and

*Trichoderma viride.* Bassuny *et al.* (2003) found that rice and bean straw treated with biological treatment significantly (P<0.05) improved IVDMD and IVOMD. Singh (2004) found that 4% urea and fungi when treated with wheat and rice straw, the values in IVDMD and IVOMD were significantly higher for urea treated straw (P<0.01) than untreated and fungi treated straw.

The results obtained of chemical composition, cell wall constituents, SEM and *in vitro* evaluation suggested that the best treatments detected were urea, fungi and fungi + bacteria + urea which were used in *in vivo* metabolism trials in comparison with untreated bagasse and hay rations.

 Table (5): Effect of chemical, biological and biochemical treatments on *in vitro* DM and OM disappearance of sugarcane bagasse

Item	IVDMD, %	±SE	IVOMD, %	±SE
T <sub>1</sub> : Unt.	12.00 <sup>g</sup>	1.73	18.00 <sup>h</sup>	1.155
Chemical treatment :				
T <sub>2</sub> :Urea Tr.	25.33 <sup>ab</sup>	1.45	32.0 <sup>abc</sup>	1.15
Biological treatments:				
T <sub>3</sub> :F Tr.	17.27 <sup>ef</sup>	0.696	33.23 <sup>a</sup>	0.710
T <sub>4</sub> :B Tr.	14.26 <sup>fg</sup>	1.27	32.60 <sup>ab</sup>	1.50
T <sub>5</sub> :Y Tr.	18.35 <sup>e</sup>	1.357	21.54 <sup>gh</sup>	0.889
T6:F + Y Tr.	17.06 <sup>ef</sup>	1.07	21.00 <sup>gh</sup>	1.155
$T_7$ :F + B Tr.	22.70 <sup>bc</sup>	1.097	24.00 <sup>fg</sup>	1.155
$T_8:B + Y Tr.$	17.27 <sup>er</sup>	0.696	27.87 <sup>de</sup>	1.657
Overall mean of biological treatments	17.82	-	26.71	-
Biochemical treatments:				
$T_9$ :F + urea Tr.	$22.40^{bcd}$	0.231	25.40 <sup>ef</sup>	1.38
$T_{10}$ :B + urea Tr.	25.40 <sup>ab</sup>	1.386	28.60 <sup>cde</sup>	1.15
$T_{11}$ : Y + urea Tr.	20.10 <sup>cde</sup>	1.039	20.00 <sup>h</sup>	0.981
$T_{12}$ :F + Y + urea Tr.	20.40 <sup>cde</sup>	0.693	26.20 <sup>def</sup>	1.154
$T_{13}$ :F + B + urea Tr.	26.60 <sup>a</sup>	0.751	29.43 <sup>bcd</sup>	0.652
$T_{14}$ :B + Y + urea Tr.	19.20 <sup>de</sup>	0.751	20.30 <sup>h</sup>	0.577
Overall mean of biochemical treatments	22.35	-	24.99	-

a, b,c,d,e,f,g and h : Means in the same column with different superscripts are different at (P<0.05).

### Effect of different treatments on digestion coefficients and nutritive value of growing lambs:

Results of nutrients digestion coefficients and nutritive values of the experimental rations are shown in Table (6). Digestibility coefficients of the experimental rations indicated significant differences (P<0.05) for different nutrients, except DM and EE which showed insignificant differences. As for OM,  $R_4$  (bagasse treated with fungi) indicated higher OM digestibility value compared with different experimental bagasse rations and without significant difference with  $R_5$  (biochemical treated bagasse). However, different experimental bagasse rations indicated lower OM digestibility compared with the hay group. The similar trend also observed with both of CP and CF, since  $R_4$  indicated higher (P<0.05) digestibility values and without significant difference with  $R_5$ . The improvement in digestibility coefficient of CF, CP and DM may be due to the improvement in chemical composition, especially in CP and CF as a result of biological and biochemical treatments. These results are in a good agreement with those obtained by **Agosin** *et al.* (1986) who indicated that *Phanerochaete chrysosporium* increased DM digestibility.

The improvement in nutrients digestibility are associated with increasing the digestion of fibrous materials, particularly hemicellulose, in addition to the increased bacterial digestion of cell wall content (Hassan *et al.*, 2005).

Table (6): Nutrients digestibility and nutritive values of different experimental rations (%)

		Expe	erimental rati	ons			
Item	<b>R</b> <sub>1</sub>	<b>R</b> <sub>2</sub>	<b>R</b> <sub>3</sub>	R 4	R <sub>5</sub>	±SE	Sig.

Digestibility, %:							
DM	91.42	86.42	85.37	88.38	87.23	0.80	NS
ОМ	80.25 <sup>a</sup>	72.29 <sup>c</sup>	70.33 <sup>c</sup>	76.18 <sup>b</sup>	73.81 <sup>bc</sup>	1.35	*
СР	72.22 <sup>a</sup>	58.18 <sup>c</sup>	60.95°	64.81 <sup>b</sup>	62.65 <sup>bc</sup>	1.31	*
CF	77.77 <sup>a</sup>	58.81 <sup>b</sup>	63.73 <sup>b</sup>	76.21 <sup>a</sup>	73.57 <sup>a</sup>	1.88	*
EE	85.60	86.57	87.56	83.34	86.25	0.987	NS
NFE	85.84 <sup>a</sup>	72.09 <sup>c</sup>	75.60 <sup>b</sup>	78.64 <sup>b</sup>	76.21 <sup>b</sup>	2.28	*
NDF	62.92 <sup>a</sup>	49.98 <sup>b</sup>	62.62 <sup>a</sup>	63.57 <sup>a</sup>	62.63 <sup>a</sup>	0.29	*
ADF	55.63°	31.78 <sup>e</sup>	60.27 <sup>b</sup>	71.32 <sup>a</sup>	50.10 <sup>d</sup>	3.01	*
Hemicellulose	75.74 <sup>b</sup>	71.25 <sup>c</sup>	66.54 <sup>d</sup>	50.57 <sup>e</sup>	83.69 <sup>a</sup>	0.57	*
Cellulose	57.61 <sup>b</sup>	36.35°	58.06 <sup>b</sup>	71.69 <sup>a</sup>	58.49 <sup>b</sup>	2.63	*
Nutritive value, 9	% on DM:						
TDN	81.00 <sup>a</sup>	68.99°	72.16 <sup>c</sup>	76.52 <sup>b</sup>	76.29 <sup>b</sup>	0.823	*
DCP	8.75 <sup>a</sup>	5.18 <sup>c</sup>	6.05 <sup>b</sup>	6.32 <sup>b</sup>	6.47 <sup>b</sup>	0.281	*

NS: Non significant difference. \*: Significant difference at (P<0.05).

a, b,c,d and e : Means in the same row with different superscripts are different at (P<0.05).

Consequently, it could be concluded that biological treatment with fungi and the mixture of fungi + bacteria + urea as a biochemical treatment could increase the digestibility coefficients of most nutrients compared with the untreated one. These results are in agreement with those reported by several researcher worked on stalks like Azzam (1992) and Singh and Gupta (1990). Mahrous (2005) who stated that the biological treatment could be used successfully to enrich poor quality roughages (cotton stalks) and improved the nutritive value (digestibility coefficients and feeding value).

Zewil (2005) showed that almost nutrient digestibilities were improved with *Trichoderma viride* (fungus) treatment in sheep rations. Similar results were obtained by Deraz (1996), El-Kady et al. (2006) and Allam et al. (2006) who found that animals fed biologically treated roughages were the most efficient groups followed by those fed chemically treated roughages.

Concerning the NDF, different treated bagasse rations indicated insignificant digestibility differences compared with the hay ration ( $R_1$ ). And all were higher (P<0.05) than that of the untreated bagasse ration which recorded the lower (P<0.05) NDF digestibility.

Acid detergent fiber digestibility showed higher (P < 0.05) value with fungus treated bagasse  $(R_4)$ compared with different treated bagasse rations and the hay ration. On the contrary, the untreated bagasse ration (R<sub>2</sub>) recorded the lower (P<0.05) ADF digestibility value. The improvement in fiber fraction as a result of biological and biochemical treatments may be due to the effect of the cellulose enzyme of fungi, which may be responsible for the stepwise hydrolysis of cellulose to glucose. These results are in agreement with those obtained by Abdel-Malik et al. (2003) who reported that, digestibility coefficients of DM, OM, CP, CF, NFE, NDF, ADF, ADL, hemicellulose and cellulose were increased significantly for banana by-products treated with both chemical (urea or acid plus urea ) and biological

treatment (bacteria, fungi, or bacteria plus fungi) than those untreated. On the other hand, Khorshed (2000) reported that all biological treatments (T.viride, S. cereviciae or T. viride + S. cereviciae)significantly (P<0.05) increased apparent nutrients digestibility for DM, OM, CP, CF, NFE, NDF, ADF, ADL, hemicellulose and cellulose than their control. During alkali treatments of lignocellulosic materials the hemicelluloses are found to be partly solubilised and more accessible to microbial digestion by alkali swelling effect (Thander and Aman, 1984). It is also indicated that, xylans are partly translocated during alkali treatment to a position in straw material where they are more available to rumen digestion (Lindberg et al., 1983). These highest digestibilities in NDF and ADF caused by fungi treatment are encouraging because they imply that the degradability and intake of sugarcane bagasse may be improved by chemical treatment.

As for hemicellulose, the biochemical treatment (R<sub>5</sub>) indicated higher (P<0.05) digestibility value in comparison with chemical  $(R_3)$  and biological treated bagasse  $(R_4)$ . The hay control ration and the untreated bagasse recorded higher (P<0.05) values in comparison with R<sub>3</sub> and R<sub>4</sub> but lower (P<0.05) than that of  $R_5$ . These results agree with those obtained by Silva and Ørskov (1988) who reported that treatment of straw with urea increased its nutritional value by making more digestible cellulose and hemicellulose available. This creates favorable conditions in the rumen for the developments of the cellulytic bacteria which best degrade the cell wall. Moreover, treatment with urea increased the degradable fractions of the straw, as well as the speed of degradation (Nandra et al., 1983 and Ibrahim et al., 1989).

As for cellulose digestibility,  $R_4$  (bagasse treated with fungi) recorded highest (P<0.05) value compared with different treated bagasse rations and the hay ration. The improved digestibility resulting from the treatments is due to two causes: (a) the solubilization of the hemicellulose (Laurent *et al.*,

**1982 and Horton, 1983)**, and (b) the alteration of the crystalline structure of cellulose (favoring the action of microorganisms) owing to an indirect effect **(Han** *et al.*, **1983)**.

As for the nutritive value (Table, 6), the hay ration recorded highest (P<0.05) nutritive values in terms of TDN and DCP compared with the different treated and untreated bagasse rations. These results may be due to the higher crude fiber content in untreated bagasse. On the other hand, these results indicated that the inclusion of treated bagasse at 30% in place of berseem hay did not improve the nutritive value of sheep rations .The highest TDN and DCP values were recorded with R<sub>1</sub> (berseem hay +CFM), while, the lowest values were recorded with R<sub>2</sub> (untreated bagasse).

Such results may suggest that different treatments led to improve the nutritive value of treated bagasse. However, both of the fungus treatment and the biochemical ones were more pronouncly effective in improving the nutritive value of bagasse rather than the chemical treatment in terms of TDN and DCP.

Such improvement was as high as 11% TDN and 25% DCP compared with the untreated bagasse (raw material). Results of the present study are in agreement with those obtained by **Abd El-Galil** (2000),who found that the biological treatment of bagasse increased TDN value significantly (P<0.05) from 46.5 to 68.9 %. **El-Ashry** *et al.* (1997) found that TDN value increased from 63.93 and 63.35% in untreated rice straw and corn stalks to 72.31 and 72.88% in fungal treated ones, respectively. Ward and Perry (1982) compared ground corn cobs treated with cellulase from *T. viride* and untreated in diet of lambs. They found an increase in digestibility of DM (4.8%), NFE (9%) and TDN (18%) and a decrease in digestibility of CF (41%) and EE (8.8%) compared with untreated one.

### Effect of different treatments on nitrogen balance of growing lambs:

Data presented in Table (7) illustrated dietary nitrogen utilization by sheep fed the different experimental rations.

Data obtained indicated significant differences among different experimental groups in different N terms. As shown highest (P<0.05) total nitrogen intake (TNI) was observed with hay group ( $R_1$ ), but without significant difference with different treated bagasse rations. The lowest (P<0.05) TNI was observed with untreated bagasse ration (R2).

Nitrogen balance showed that all rations achieved positive N balance. However, the hay control group ( $R_1$ ) retained more (P<0.05) N, but without significant difference with R4. Both the two groups indicated higher (P<0.05) N balance in compare with different treated and untreated bagasse rations. The variability in dietary nitrogen retained might be due to its escape from ruminal fermentation or may probably due to an increased utilization of ammonia in the rumen (Holzer *et al.*, 1986).

Item		Ex]	perimental ra	tions			
Item	<b>R</b> <sub>1</sub>	R <sub>2</sub>	<b>R</b> <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	±SE	Sig.
N. intake g/h/d	27.12 <sup>a</sup>	18.11 <sup>b</sup>	24.24 <sup>a</sup>	24.89 <sup>a</sup>	23.86 <sup>a</sup>	0.887	*
Fecal N g/h/d	12.86 <sup>ab</sup>	9.09 <sup>c</sup>	14.26 <sup>a</sup>	12.09 <sup>b</sup>	12.32 <sup>b</sup>	0.496	*
Urinary N g/h/d	7.37 <sup>ab</sup>	6.67 <sup>ab</sup>	6.14 <sup>b</sup>	8.47 <sup>a</sup>	7.48 <sup>ab</sup>	0.304	*
Total excreted N g/h/d	20.23 <sup>a</sup>	15.76 <sup>b</sup>	20.40 <sup>a</sup>	20.56 <sup>a</sup>	19.80 <sup>a</sup>	0.588	*
N digested g/h/d	14.26 <sup>a</sup>	9.02 <sup>c</sup>	9.98 <sup>bc</sup>	12.80 <sup>ab</sup>	11.54 <sup>abc</sup>	0.633	*
N balance g/h/d	6.89 <sup>a</sup>	2.35 <sup>b</sup>	3.84 <sup>b</sup>	4.33 <sup>ab</sup>	4.06 <sup>b</sup>	0.505	*
N balance % intake	25.41 <sup>a</sup>	12.98 <sup>b</sup>	15.84 <sup>ab</sup>	17.40 <sup>ab</sup>	17.02 <sup>ab</sup>	1.718	*
N balance % digested	48.32 <sup>a</sup>	26.05 <sup>b</sup>	38.48 <sup>ab</sup>	33.83 <sup>ab</sup>	35.18 <sup>ab</sup>	2.908	*

 Table (7): Nitrogen balance of lambs fed rations containing untreated and treated sugarcane bagasse

\*: Significant difference at (P<0.05).

a, b and c: Means in the same row with different superscripts are different at (P<0.05).

As a general conclusion, dietary nitrogen utilization favored  $R_4$  (biological treated bagasse ration) followed by  $R_5$  (biochemical Tr. bagasse ration) as the most efficient groups in utilizing the dietary N of the treated bagasse rations. Such results indicated the effective role of both the fungus and biochemical treatments in improving dietary N utilization by experimental animals. And although, the biological treatment ranked first in compare with different applicable treatments in improving N utilization by experimental animals; however, different treated bagasse rations ranked in second category to that of the hay ration ( $R_1$ ) i.e. conventional roughage. These results were in harmony with those obtained by Langer *et al.* (1982) and Ahuja *et al.* (1986) who found that ration containing biologically treated crop-residues showed positive nitrogen balance. According to Mohamed (2005) and Allam *et al.* (2006) with sugar beet pulp and Deraz and Ismail (2001) with biologically treated cotton stalks, diets contained 30% biologically treated sugar beet pulp showed higher (P<0.05) N balance than the control.

### Conclusion

The present study suggested that the possibility of improving the chemical composition, nutritive value and nitrogen utilization of sugarcane bagasse by different chemical, biological and biochemical treatments in rations for lambs. The superiority effect of fungi or fungi + bacteria +urea treatments was observed compared with urea treatment.

Further work is needed to study the effect of using untreated or treated sugarcane bagasse by different treatments in place of berseem hay in rations for growing lambs on their performance.

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