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EFFECT OF BACTERIAL TREATMENTS ON CHEMICAL COMPOSITION, CELL WALL CONSTITUENTS AND DIGESTIBILITY OF RICE STRAW.

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SUMMARY

This study investigated the effect of treatments by cellulolytic bacteria (*Ruminococcus albus* and *Clostridium cellulovorans*) on rice straw in anaerobic condition. Two experimental trials were conducted; first experiment: bacteria were used as biological treatments (T1: rice straw untreated, T2: rice straw treated by *Ruminococcus* and T3: rice straw treated by *Clostridium*) to study the changes that occur in chemical composition and cell wall constituents of ensiled rice straw. Silage was made from rice straw and incubated for two months and investigated effect of treatments by the two strains of bacteria on IVDM and IVOM disappearance of silage. Second experiment: in complete randomized design with 21days period, twelve Barki sheep about 32±0.5 kg body weights were fed on three rations (R1: 70% rice straw untreated +30%sweet potatoes, R2:70%rice straw treated by *Ruminococcus*+30%sweet potatoes and R3: 70%rice straw treated by *Clostridium*+30%sweet potatoes) to study effect of treatments on digestibility, nitrogen balance, TDN, some rumen and blood parameters. The results indicated that using bacteria caused increase in crude protein (from 1.45 to15.16%) and decrease in crude fiber (from 44.08 to 28.44%) of rice straw. The two treatments significantly ($P<0.05$) decreased NDF, ADF, cellulose and hemicellulose. In addition, the bacterial treatments by *Ruminococcus albus* and *Clostridium cellulovorans* succeeded with rice straw. However, treatments increased IVDMD, IVOMD and digestibility ($P<0.05$) compared with the untreated rice straw, But rumen and blood parameters were not significantly different ($P>0.05$) between the two treatments. In addition, the bacterial treatments by *Ruminococcus albus* and *Clostridium cellulovorans* succeeded with rice straw which increased TDN from 54.95% in untreated rice straw (R1) to 70.08 and 69.02% in treated rice straw by *Ruminococcus* (R2) and *Clostridium* (R3), respectively. These results suggest that bacterial treatments by *Ruminococcus albus* and *Clostridium cellulovorans* as silage improved digestibility and nutritive value of rice straw.

Keywords: *bacteria, rice straw, cell wall constituents, nutritive value, sheep.*

INTRODUCTION

Rice straw, the major roughage of ruminants in Egypt, contains very little protein and mineral and very high amount of lignin and silica. The lignin component creates the barrier to efficient utilization, conversion or degradation of the polysaccharides in lignocelluloses to useful products. Lignocellulosic residues are not high value feeds, they are classified as low quality roughage, i.e. high in fiber, low in protein, vitamins and minerals and high lignifications make them less utilization than the green fodder. Microbiological treatments of the cellulosic materials have been tried for improving the nutrient availability from such materials to the animals (Hunt *et al.*, 1992 and Singh *et al.*, 1993). Use of straw for ruminants is mainly constrained by its poor nutritive value, since it has a low energy and N content and low intake. During the last 20 years, there has been a great interest in developing methods to improve straw and other low digestibility roughages, by supplementation and physical or chemical treatments (Sundstol and Owen, 1984). The same authors, in a previous study, found that biological treatment by *Cellulomonas sp.* of bagasse improved its nutritive value (TDN = 69%, approximately equal to the nutritive value of corn) by increasing the crude protein from 1.7% to 15.5%. In addition, these bacteria were very active to secrete the cellulase enzymes causing degradation of cell wall constituents of bagasse and decrease the crude fiber from 44.9 % to 30.6% (Abd El-Galil, 2000). Abd El-Galil (2006) found values of nutrient digestibility coefficient of 77.29% ,79.47%, 84.44 %, 70.87%, 86.13, 88.36, 80.33 and 94.28 for CP ,CF ,EE , NFE, NDF, ADF, Hemicellulose and cellulose , respectively ,when she used treated corn stalks with *Ruminococcus albus* up to 30 % in sheep rations . This study aimed to investigate the effect of bacterial treatments with *Ruminococcus albus* or *Clostridium cellulovorans* on rice straw as agricultural by-products on chemical composition, cell wall constituents, IVDMD, IVOMD, nutrient digestibility, nitrogen balance and some ruminal and blood parameters of sheep.

MATERIALS AND METHODS

The present study was divided into two Experiments:

First experiment:

Isolation of bacteria:

Two strains of cellulolytic bacteria were prepared to treatment rice straw. The separated strains are Ruminococcus albus and Clostridium cellulovorans. The isolation of species used streak-plate or the pour-plate method. The pour-plate technique for the isolation of pure cultures was conducted according to A.T.C.C. (1992). A liquid suspension of the microbe was diluted serially in melted nutrient agars that supported the growth of the microbe. Agar at 45°C remains liquid but does not kill microbes. Each dilution in the series contains a lower concentration of the microbes per milliliter, so individual bacteria are spatially separated from one another in the liquid. The agar for each dilution is poured into Petri dishes, and was allowed to solidify, and then the plates were incubated at 38°C. Bacteria suspended in the milted agar were trapped in the agar as it solidified. During incubation, the isolated bacteria develop into visible colonies wherever the bacteria had become localized in the agar or on the agar surface. At the higher

concentration dilutions, in which bacteria are physically separated from each other, discrete colonies are observed.

Ensiling (small scale silo study):

Rice straw was sun dried to 90% DM and chopped to 1-3 cm and mixed with water, molasses, urea, formic acid and acetic acid according to Abd El-Galil (2000). The samples were treated with one of the following treatments by 2 liters (6×10^5 viable anaerobes/kg of wet silage) /ton: T1:- Untreated rice straw. T2:- treatment with *Ruminococcus albus*. T3:- treatment with *Clostridium cellulovorans*. Treated samples were pressed in 2 liters jars for laboratory use or barrels (200 liters capacity) for farm use and incubation for two months.

In Vitro dry matter and organic matter disappearance:

The In Vitro dry matter (DM) disappearance and organic matter (OM) disappearance were determined according to the method described by Terry *et al.* (1969). Two tubes, as replicates for each sample, were used at different incubation times (2, 4, 6, 24 and 48 hrs.).

Second experiment

The metabolism trial:

A metabolism trial was carried out to evaluate the nutrient digestibility and nitrogen balance (NB) using sheep that fed the experimental rations. The complete randomized block design (CRD) was used for testing this experiment. Twelve mature Barki sheep (32± 0.5 kg. wt.) were used in this experiment. Each animal was confined in an individual metabolic crate for 14 days as an adaptation period followed by five days as a collection period. Animals were fed at maintenance level requirements using the allowances of NRC (1985).

The animals were distributed to three similar groups (4 animals per each ration) and were fed as follows:

R1: 30% sweet potatoes + 70% untreated rice straw.

R2: 30% sweet potatoes + 70% treated rice straw with *Ruminococcus albus*.

R3: 30% sweet potatoes + 70% treated rice straw with *Clostridium cellulovorans*.

Rumen liquor samples were taken from each animal at the end of the collection period at 0, 2, 4 and 6 hours after morning feeding using a rubber stomach tube. The pH was determined using a pH-meter (EIL-7010) with a combination electrode. Ammonia nitrogen (NH3-N) was immediately determined (Conway, 1963). Frozen rumen liquor samples were analyzed for total volatile fatty acids (TVFA's) by steam distillation according to Warner (1964). Blood samples were collected from three animals per group at the end of metabolism trial at zero, 2, 4 and 6 hrs. post feeding. Samples were obtained by allowing blood to flow freely from the jugular vein through heparin tubes. Then, centrifuged for 30 min. at 4000 rpm, plasma was separated into clean dried glass vials and stored at freeze(-20°C) till analysis. Blood plasma was analyzed using special kits for urea (Patton and Crouch, 1977), total protein (Henry, 1964) and creatinine (Henry, 1974).

Proximate analysis:

The proximate analysis of treatments, experimental rations, feces and urine were determined according to A.O.A.C. (1990). The proximate analyses were used to determine dry matter (DM), crude protein (CP), crude fiber (CF), Ether Extract (EE) and ash. The

nitrogen free extract (NFE) was obtained by the difference.

Cell wall constituents analysis:

Raw rice straw compost treated and untreated rations and feces were analyzed according to Van Soest and Breston (1979) to determine neutral detergent fiber (NDF), Acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose, cellulose and lignin were determined by the difference.

Statistical analysis:

The data of chemical composition, cell wall constituents, in vitro (DM and OM), in vivo, rumen and blood parameters were statistically analyzed according to Statistical Analysis Systems User's Guide, (S.A.S., 1998). Separation among means was carried out by using Duncan Multiple test, (Duncan, 1955). The following model was used:

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

Where:

Y ij = the observation of the model.

μ = General mean common element to all observation.

Ti = the effect of the treatment (i = 1, 2, 3)

α ij = The effect of error.

RESULTS AND DISCUSSION

First experiment

Effect of bacterial treatments on rice straw:

Chemical composition and cell wall constituents of treated and untreated rice straw are presented in Table (1). The present data indicated that the value of crude protein increased for treated rice straw which in T2 was 15.16 % and in T3 was 12.45% compared to untreated rice straw (T1). While the treated rice straw with *Ruminococcus albus* (T2) had the highest value. These effects might be due to nitrogen content of the added urea (about 3% w/w), the microbial protein from bacteria treatments and the nitrogen content of growing cellulolytic bacteria in silage of rice straw. These results agreed with those noted by Zhao *et al.*, (2002) and Abd El- Galil (2006). However, it can be suggested that N content increased due to contamination by water, soil or plastic container. In addition, (Denek and Deniz, 2004) cited that the addition of urea only into maize silage decreased DM digestibility. It can be concluded that urea and molasses can be used as silage additive to increase crude protein and energy content of silage.

Crude fiber decreased from 44.08% in untreated rice straw to 32.32% and 28.44 % in treated T2 and T3, respectively. But treated rice straw with *Clostridium cellulovorans* (T3) was highly effected CF content compared to other treatments .The decrease of crude fiber values in the experimental treatments could be a results of the cellulase enzymes (secreted by cellulolytic bacteria) effect. It is clear that ash values increased from 14.09% in untreated rice straw (T1) to 13.68% and16.20 % when treated by *Ruminococcus albus* (T2) and *Clostridium cellulovorans* (T3), respectively. These effects might due to the added media of growing bacteria, molasses and strains of cellulolytic bacteria used for treating rice straw. In general , it can be concluded that bacterial treatments had great effect on increasing crude protein content (from 1.45 to 15.16%) and increasing crude fiber degradation (from 44.08 to 28.44 %) of rice straw. The present finding is in agreement with Bakshi and Langer (1991). They reported that crude fiber decreased from 42.92 to 17.87 % in the compost and spent with the treatment of cellulase enzymes .On the same trend, supportive results were reported by Eduardo and Etienne (1985); Larwence and

Abada (1987) and Abd El- Galil (2006). The present results confirm the results obtained by Abdul- Aziz *et al.* (1997). Gado (1999) fermented rice straw and bagasse with *Trichoderma ressei* and reported that the CP, CF, EE, NDF, ADF, cellulose and hemicellulose were lowered significantly (P<0.05) in both treated rice straw and bagasse. Bader (2001) found that the biological treatments of corn stalks by *P. florida* decreased CF content than that in untreated corn stalks, being 3.5%, while combined fungi and bacteria at level of 3% were more efficient in decreasing crude fiber content from 37.85% to 18.42%, followed by incubated of corn stalks with *P. florida* and *E. carotovora* at level 2% (being 47.37% of control). Gado *et al.*(2007) found that treatments with solution (S) which contained urea , molasses , formic acid and acetic acid added to bagasse compared control(U), cellulase enzymes(E), rumen liquor (R) and *Cellulomonas cellulasea* (B) increased crude protein without a decrease in crude fiber and cell wall constituents.

Table (1): Effect of bacterial treatments on chemical composition and cell wall constituents of rice straw (% on DM basis).

Item	T1 (Untreated)	T2 (Ruminococcus albus)	T3 (Clostridium cellulovorans)	±S.E
DM	90.05 ^{a}	89.89 ^{a}	87.90 ^{b}	± 0.178
OM	85.91 ^{a}	86.32 ^{a}	83.79 ^{b}	± 0.190
CP	1.45 ^{c}	15.16 ^{a}	12.45 ^{b}	± 0.112
CF	44.08 ^{a}	32.32 ^{b}	28.44 ^{c}	± 0.157
EE	2.21 ^{a}	2.33 ^{a}	2.84 ^{a}	± 0.039
NFE	38.18 ^{a}	36.49 ^{b}	40.06 ^{c}	± 0.371
Ash	14.09 ^{b}	13.68 ^{b}	16.20 ^{a}	± 0.050
NDF	77.09 ^{a}	63.53 ^{b}	60.75 ^{c}	± 0.191
ADF	47.28 ^{b}	35.59 ^{c}	49.94 ^{a}	± 0.220
ADL	10.89 ^{b}	15.74 ^{a}	14.25 ^{a}	± 0.180
Hemicellulose	29.81 ^{a}	27.94 ^{b}	10.85 ^{c}	± 0.230
Cellulose	36.39 ^{a}	19.85 ^{b}	35.65 ^{a}	± 0.310

- *a* , *b* and *c* Means within the same row with different superscripts differ significantly (P < 0.05)

Bacterial treatments decreased (P<0.05) NDF contents from 77.09 % in untreated rice straw (T1) to 63.53 % and 60.75% in T2 and T3, respectively. The highest decrease in NDF content was recorded in T3. There were significant differences (P<0.05) among treatments detected in ADF content from 47.28% in untreated (T1) to 35.59 % in treated T2 which treated with *Ruminococcus* that was more effective on ADF content. On the other side, it was noticed that hemicellulose content decreased in T2 (27.94%) and T3 (10.85%) compared to untreated raw (29.81%) while treatment with *Clostridium* (T3) had the highest decrease. It was noticed that bacterial treatments decreased cellulose content in T2 (19.85%) compared to other treatment where bacteria degraded the bonding between cellulose and other components. In all bacterial treatment we found that the straw treated with cellulolytic bacteria increased the lignin content. In general, results indicated that bacterial treatment with two cellulolytic strains of rice straw had significant effect on cell wall constituents with treatment by *Ruminococcus* was more effect on ADF and cellulose content while treatment by *clostridium* was more effective on NDF and hemicellulose content. Bader (2001) reported that biological treatments effective with fungi decreased (P<0.05) NDF, ADF, ADL, cellulose and hemicellulose in rice straw and corn stalks. Autrey *et al.*(1975) reported a 13% decrease in the cellulose content of corn silage when it was treated with cellulase enzyme. Also, Spoelstra *et al.*(1992) and Stockes and

Chen (1994) reported a reduction of NDF value after enzyme addition to corn silage.Also. Sheperd and Kung (1996) found that enzyme treatment to whole plant corn silage (WPCS) reduced NDF and ADF contents. In addition, Wang *et al.* (2002) cited that the treatments of corn stalks with microbial inoculants (209 gm lactic acid bacteria or *lactobacillus sp.*) and enzyme preparation 400 gm/100kg DM(100gm cellulase +200gm amylase +100gm glucoamylase), and increased the degradable DM and NDF compared with control (untreated).

In Vitro dry matter disappearance (IVDMD) and In Vitro organic matter disappearance (IVOMD) of rice straw:

Data in Table (2) exhibited the effect of bacterial treatments on In Vitro dry matter and organic matter disappearance of rice straw. After 48 hrs fermentation the highest significant ($P<0.05$) increase for IVDMD was found in treated rice straw with *Clostridium cellulovorans* (89.71 %), while the lowest significant increase value was found in the untreated rice straw(59.01%). After 2 hrs, it was noticed that IVDMD increased in rice straw treated with *Ruminococcus albus* (15.11%) compared to other treatments. Also, after 4 hrs, 6 hrs, 24 hrs and 48 hrs recorded increase for IVDMD in treated rice straw with *Clostridium cellulovorans* (21.33 %, 40.32%, 55.47 % and 89.71%) compared to other treatments. Bacterial treatments with *Ruminococcus albus* and *Clostridium cellulovorans* increased ($P<0.05$) IVOMD of rice straw incubated for 48 hrs than that in untreated one. After 2hrs, 4hrs and 48hrs, it was noticed that IVOMD increased in rice straw treated with *Ruminococcus albus* (13.98%, 20.53% and 80.52%) compared to other treatments. Also, after 6 hrs and 24 hrs recorded increase for IVOMD in treated rice straw with *Clostridium cellulovorans* (40.39% and 53.11%) compared to other treatments. Generally, the increase in IVDMD and IVOMD of biologically treated rice straw as poor quality roughage may be due to the decreases of the amount of CF, NDF, ADF and increases in CP content. It is well documented that dry matter digestibility is positively correlated to CP content and negatively correlated to CF, ADF and NDF (Sawe *et al.*, 1998). These results are in a good agreement with results obtained by Abdul –Aziz *et al.* (1994). Also, the present results are in harmony with the findings of (Gado 1999 ; Abd El-Galil 2000 & 2006 and ; Bader 2001).

Table (2): Effect of bacterial treatments on invitro dry and organic matter disappearance of rice straw (% on DM basis).

Item	T1 (Untreated)	T2 (Ruminococcus albus)	T3 (Clostridium cellulovorans)	±SE
IVDMD (%)				
2hrs	10.35^{c}	15.11^{a}	14.23^{b}	±1.21
4hrs	15.44^{c}	19.27^{a}	21.33^{b}	±1.21
6hrs	32.63^{c}	37.16^{b}	40.32^{a}	±1.21
24hrs	45.82^{c}	49.58^{b}	55.47^{a}	±1.21
48hrs	59.01^{c}	78.31^{b}	89.71^{a}	±1.21
IVOMD (%)				
2hrs	5.44^{c}	13.98^{a}	11.65^{b}	±0.99
4hrs	12.34^{c}	20.53^{a}	19.49^{b}	±0.99
6hrs	23.53^{c}	32.75^{b}	40.39^{a}	±0.99
24hrs	35.66^{c}	44.56^{b}	53.11^{a}	±0.99
48hrs	50.97^{c}	80.52^{a}	76.80^{b}	±0.99

- *a* , *b* and *c* Means within the same row with different superscripts differ significantly ($P < 0.05$)

Second experiment:

Digestion coefficients (%) of rice straw:

It is clear from Table (3) and Table (4) that treatments by cellulolytic bacteria had a significant effect on digestibility of the rice straw. It was observed that the digestibility coefficient of OM in sheep fed treated rice straw with *Ruminococcus albus* (R2) and *Clostridium cellulovorans* (R3) increased from 73.06% in untreated(R1) to 87.75 % and 82.93 %, respectively.

Also, crude protein digestibility of treated rice straw increased to 61.87% and 80.14% in R2 and R3 respectively, compared to that of R1 (25.68 %). Data indicated that crude fiber digestibility of untreated rice straw was 55.01%, while in treated rice straw were 87.80% and 86.63% when rice straw treated by *Ruminococcus albus* and *Clostridium cellulovorans* respectively. However, in R3 which treated with *Clostridium* the value of NFE digestibility was increased from 75.84% in untreated (R1) to 90.15%. Generally, the results showed that bacterial treatments positively affected the digestibility of nutrients of treated rice straw. Data showed that NDF digestibility of untreated rice straw was 69.04% while that of treated with *Ruminococcus* (R2) was increased to 76.12 %. In addition, treatment with *Clostridium cellulovorans* affected on NDF digestibility significantly ($P<0.05$). The ADF digestibility coefficients increased significantly ($P<0.05$) from 71.46 % in untreated to 80.48% and 83.48%in treated with *Ruminococcus albus* and *Clostridium cellulovorans*, respectively. Moreover, the hemicellulose digestibility increased from 62.37 % in untreated to 78.34% in treated with *Clostridium cellulovorans*. Also, treatment with *Ruminococcus* increased hemicellulose digestibility to 80.33%. Results showed that cellulose digestibility coefficients increased from 73.91% in untreated to 90.91% and 91.95% in treated with *Ruminococcus albus* and *Clostridium cellulovorans*, respectively.

In addition, the treatment of rice straw with *Ruminococcus* and *Clostridium* recorded a positive effect on digestion coefficients. This was due to the positive effect on cellulolytic activity of rumen microflora digestion through altering the ruminal environment.

Table (3): Chemical composition and cell wall constituents of rations used in metabolism trial.

Item	Rations			±S.E
	R1	R2	R3	
DM	100	100	100	-
OM	88.94^{a}	89.22^{a}	87.45^{b}	± 0.17
CP	2.49^{c}	12.09^{a}	10.19^{b}	± 0.10
CF	31.82^{a}	23.28^{b}	20.87^{c}	± 0.13
EE	1.90^{b}	1.98^{b}	2.34^{a}	± 0.01
NFE	52.74^{b}	51.55^{b}	54.05^{a}	± 0.28
Ash	11.06^{b}	10.78^{b}	12.55^{a}	± 0.03
NDF	56.76^{a}	47.27^{b}	45.32^{c}	± 0.16
ADF	34.29^{b}	25.79^{c}	34.16^{a}	± 0.24
ADL	7.74^{c}	11.14^{b}	10.08^{a}	± 0.19
Hemicellulose	22.47^{a}	21.48^{b}	9.15^{c}	± 0.22
Cellulose	26.55^{a}	14.65^{b}	26.08^{a}	± 0.27

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

Where, Chemical composition of sweet potatoes was 93.80, 4.94, 3.20, 1.15, 86.7, 4.0, 9.24, 4.0 and 0.38% for DM, CP, CF, EE, NFE, Ash, NDF, ADF and ADL, respectively.

R1: 30% potatoes + 70% untreated rice straw.

R2: 30% potatoes + 70% treated rice straw with *Ruminococcus albus*.

R3: 30% potatoes + 70% treated rice straw with *Clostridium cellulovorans*.

Table (4): Effect of bacterial treatments on digestion coefficients in sheep fed treated or untreated rice straw (%on DM basis).

Item	Rations			±S.E
	R1	R2	R3	
DM	69.61 ^{b}	82.50 ^{a}	81.61 ^{a}	± 0.20
OM	73.06 ^{c}	87.75 ^{a}	82.93 ^{b}	±0.21
CP	25.68 ^{c}	61.87 ^{b}	80.14 ^{a}	±0.29
CF	55.01 ^{b}	87.80 ^{a}	86.63 ^{a}	±0.19
EE	62.43 ^{b}	88.14 ^{a}	87.11 ^{a}	±0.33
NFE	75.84 ^{b}	89.60 ^{a}	90.15 ^{a}	±0.35
NDF	69.04 ^{c}	76.12 ^{b}	80.21 ^{a}	±0.31
ADF	71.46 ^{c}	80.48 ^{b}	83.48 ^{a}	±0.27
Hemicellulose	62.37 ^{c}	75.84 ^{b}	78.34 ^{a}	±0.34
Cellulose	73.91 ^{b}	90.91 ^{a}	91.95 ^{a}	±0.26
TDN	52.30 ^{b}	70.08 ^{a}	71.02 ^{a}	±0.35
N balance, g	+ 3.26 ^{b}	+ 9.81 ^{a}	+ 10.07 ^{a}	±0.19

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

In general, the treatment of rice straw by *Clostridium* (R3) improved cell wall constituents digestibility compared with untreated. Ahuja *et al.,* (1986) found that values of nutrients digestibility were 61.6, 66.7, 48.7, 63.7 and 71.4 % for DM, CP, CF, EE and NFE when they fed treated wheat straw with fungi up to 30 % of sheep ration. These results are in agreement with results obtained by (Abd El-Galil 2000 & 2006).

Nitrogen balance:

Data of nitrogen balance for untreated rice straw (R1) was the lowest ($P<0.05$) value (3.26 g/d) while treated with *Ruminococcus* (R2) and *Clostridium* (R3) were increased to 9.81g/d and 10.07 g/d, respectively, this mean that the treatment rice straw improved nitrogen balance. The increase in N-balance in R2 and R3 may be due to the possible increased production of microbial protein synthesis or increased presence of fermentable energy (Tagari *et al.* 1976), the variability in nitrogen that might escape ruminal fermentation or an increased utilization of ammonia in the rumen (Holzer *et al.* 1986).

Feeding values:

It is noticed that there were significant ($P<0.05$) differences between treatments in Table (4) where the bacterial treatment increased nutritive values of rice straw. The total digestible nutrients (TDN) of rice straw was 52.3 % in untreated (R1), while it was 70.08 % and 71.02 % in treated rice straw with *Ruminococcus albus* (R2) and *Clostridium cellulovorans* (R3), respectively. Generally, the results indicated that biological treatments had positive effects on improving nutrients digestibility and nutritive values. Abd El-Galil (2006) found that values of nutrient digestibility coefficient were 72.01 %, 77.29% ,79.47%, 84.44 %, 70.87%, 86.13, 88.36, 80.33 and 94.28 for DM ,CP ,CF ,EE , NFE. NDF, ADF, Hemicellulose and cellulose respectively, when corn stalks was treated with *Ruminococcus albus* was fed at 30 %of sheep ration . Also , results of the present study are in agreement with those obtained by Abd El-Galil (2000),who found that the biological treatments of bagasse increased TDN value significantly ($P<0.05$) from 46.5 % to 68.9 %.

Rumen liquor parameters:

Results of Table (5) indicated that rumen liquor pH values did not differ significantly (P>0.05) among treatments. It is clear that values of rumen liquor pH were also similar among sampling times for sheep fed biologically treated rice straw. The total volatile fatty acids values for treated rice straw with *Ruminococcus* (R2) and *Clostridium* (R3) were higher than that for untreated rice straw (R1), but treated rice straw with *Clostridium* had the highest value. (Mould and Orskov, 1984) indicated that the pH values always around 6.0, which that had an increase on cellulolytic bacteria contents and activity in rumen and fiber fraction digestion).The TVFA's values were high in rations R2 and R3 which may be due to a higher microbial activity in the rumen of sheep fed this rations than those received the untreated R1. El-Taweel (2000) found a positive correlation between cellulose digestion coefficient and rumen ammonia-N or TVFA's concentration. Ammonia-N values for treated rice straw with *Ruminococcus* (R2) and *Clostridium* (R3) were higher than that for untreated rice straw (R1), which treated rice straw with *Clostridium* in R3 had the highest value. These results may be due to the change in fermentation rate with advancing time after feeding in which ruminal NH3-N would satisfy microbial needs and hence maximize rate of fermentation in rumen. The present results are in agreement with those obtained by Dhanda *et al.*, (1994) and El-Ashry *et al.*, (1997), also agree with Abd El-Galil (2000) and Abd El-Galil (2006).

Table (5): Effect of bacterial treatments on ruminal parameters in sheep feeding on rice straw

Item	Rations			± SE
	R1	R2	R3	
pH				
0hrs	6.9	6.8	6.9	
2hrs	6.8	6.7	6.7	
4hrs	6.6	6.3	6.5	
6hrs	6.8	6.7	6.8	
mean	6.77^{ns}	6.62^{ns}	6.72^{ns}	± 0.100
TVFA's(m.equ./100ml)				
0hrs	9.3	10.8	11.0	
2hrs	10.4	12.8	12.9	
4hrs	11.8	14.9	15.13	
6hrs	9.9	13.4	12.18	
mean	10.35^{b}	12.97^{a}	13.00^{a}	±1.020
NH3-N(mg/100ml)				
0hrs	8.70	10.60	11.70	
2hrs	10.40	14.30	15.40	
4hrs	11.30	13.50	12.10	
6hrs	10.40	11.70	11.90	
mean	11.20^{b}	12.52^{a}	12.77^{a}	±1.011

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

Blood plasma parameters

It was noticed (Table 6) that plasma total protein increased during 0 & 6 hrs in all treatments with treated rice straw with *Ruminococcus* and *Clostridium*, but this increase

under normal level of plasma total protein in sheep. With in regard to plasma urea concentration, it was noticed to be high in all treatments at all measuring times in Table (6). There was urea concentration increased gradually from 0 hrs until 6 hrs in all treatments. Also, it is no differences between treated rice straw with *Ruminococcus* and *Clostridium,* but it differences were found between treated and untreated rice straw.

Table (6): Effect of bacterial treatments on blood parameters in sheep feeding on rice straw.

Item	Rations			± SE
	R1	R2	R3	
TP(mg/100)				
0hrs	8.90	10.40	10.90	
2hrs	10.20	11.30	12.20	
4hrs	11.50	12.40	13.60	
6hrs	12.10	12.90	11.80	
mean	10.67^{b}	11.75^{a}	12.12^{a}	±0.600
Urea(mg/100)				
0hrs	5.90	6.70	6.90	
2hrs	6.80	7.90	7.80	
4hrs	7.20	8.20	8.30	
6hrs	9.50	9.80	10.60	
mean	7.35^{b}	8.15^{a}	8.40^{a}	±0.615
Creatinine (mg/100)				
0hrs	0.70	0.80	0.90	
2hrs	0.90	1.10	1.20	
4hrs	1.00	1.20	1.10	
6hrs	0.90	1.00	1.00	
mean	0.87^{ns}	1.02^{ns}	1.05^{ns}	±0.058

- a, b and c means within the same row with different superscripts differ significantly ($P < 0.05$).

Values of plasma creatinine are shown in Table (6). The present results show no significant differences in plasma creatinine between treated and untreated rice straw. It was noticed that values of plasma creatinine were slightly increased between 0 & 4 hrs in all treatments, but after 6 hrs it decreased in all treatments. The value of creatinine was the highest in treated rice straw by *Clostridium* compared with other treatments. Generally, plasma creatinine level is a useful indicator to glomerular filtration in kidney, the values of plasma creatinine for sheep were within the normal levels. These results came on line with those obtained by Abd El-Galil (2000 & 2006).

CONCLUSION

It can be concluded that the bacterial treatments with *Ruminococcus albus* and *Clostridium cellulovorans* had the highest effect on chemical composition and cell wall constituents of treated rice straw. These results exhibited that *Ruminococcus* was more effective on cellulose but *Clostridium* was more effect on hemicellulose for rice straw compared the untreated one. The treatment by *Clostridium* increased IVDMD of rice straw.

The bacterial treatments with *Ruminococcus albus* and *Clostridium cellulovorans* could be used successfully to improve the quality of rice straw (as poor quality roughage) with increased protein, nutrients digestibility and nutritive value, with decreased fibrous fraction of rations without any adverse effects on animal performance and health.

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تأثير المعاملة بالبكتيريا على التركيب الكيميائى وتركيب جدر الخلايا و القيمة الغذائية لقش الأرز

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اجريت هذه الدراسة بقسم الانتاج الحيوانى بزراعة عين شمس بهد ف دراسة تأثير المعاملة بسلالتين من البكتبريا المحللة للسيليلولوزوهما

Ruminococcus albus and Clostridium cellulovorans

وقد اجرى البحث على مرحلتين هما:

المرحلةالاولى : تم استخدام هذه السلا لا ت المحللة للسيليلولوز (بعد تنميتها بكميا ت كافية) فى المعاملا ت البيو لوجية للسيلا ج المصنع من قش الأرز معمليا أولا لدرا سة تا ثيرها على التركيب الكيميائى وتركيب جدر خلايا قش الأرزثم ا ستخدا م قش الأرز غيرا لمعا مل والمعا مل لتقد ير قيم معا ملا ت الهضم المعملية للما دة الجا فةوالما دة العضو ية .

فى المر حلة الثانية : تم استخدا م سيلا ج قش الأرز غيرا لمعا مل والمعا مل بسلا لتين من البكتر يا كلا على حده لا جراء تجربة هضم مزرعية واستخدم فى هذه التجربة 12 ذكرا من اغنام البرقى واستمرت التجربة لمدة 21 يوما حيث تم توزيع الحيوانات بالتساوى على ثلاثة مجاميع تجريبة (4 حيوانات فى كل تجربة)كالتالى :

- المجموعةالأولى(R1) : 70 ٪ سيلاج غير معا مل +30 ٪ بطا طا .
- المجموعة الثا نية(R2): 70 ٪سيلاج معا مل ببكتيريا Ruminococcus albus +30 ٪بطا طا .
- المجموعة الثا لثة (R3): 70 ٪سيلاج معا مل ببكتيريا Clostridium cellulovorans +30 ٪بطا طا على أساس المادةالجا فة.

و تم درا سة تأثير استخدام سيلاج قش الأرز على أداء الحيو انا ت التجر يبية من خلا ل درا سة معا ملا ت الهضم وقيا س ميزان الأزوت والقيمة الغذائية وقيا س بعض المقا ييس على سا ئل الكر ش و على مكونا ت بلا زما الدم.

وأظهرت نتائج المرحلة الأولى أن رائحة السيلا ج كانت جيدة وخالية من رائحة حمض البيوتيريك والايثانول ويدل ذلك على جودة طريقة الحفظ للسيلا ج . كما لوحظ ارتفا ع نسبة البروتين الكلى فى قش الأرز المعا مل فكا نت تتراوح بين 12.45 ٪ الى 15.16 ٪ بالمقارنة بقش الأرز

غير المعامل (1.45 ٪) وكا نت متوسط نسبة الأ لياف الخام فى قش الأرز المعامل تترا وح بين 28.44 ٪ الى 32.23 ٪ با لمقا رنة بالكنترول فقد كانت44.08 ٪. وارتفعت نسبة الرماد الخا م فى القش المعا مل ببكتريا Clostridium فكانت 16.20 ٪ على أساس المادة الجا فة. واحتوى قش الأرز المعامل على نسبة NDF تتراوح بين63.53 - 60.75 ٪ بينما كانت فى الكنترول77.09 ٪. و انخفضت نسبة ADF نتيجة المعا ملا ت البكتيرية فى قش الأرز وتراوحت بين 35.59 - 49.94 ٪ بينما كانت 57.85 ٪ فى الكنترول على أساس المادة الجافة.كما لوحظ من تجربة الهضم المعملية أن المعا ملات البكتيرية لقش الأرز أدت لرفع قيمة ا لمعا ملا ت الهضمية المعملية بالمقارنة بالكنترول (غير المعامل) .

وأظهرت نتائج المرحلة الثانية أن قش الأرز المعامل بكل من and Clostridium Ruminococcus albus cellulovorans اعطى أفضل معاملات الهضم لكلا من NDF,ADF, CP ,CF, DM والهيمسليلوز والسيليلوزبالمقارنة بغيرا لمعامل.

بالنسبة لمقا ييس الكرش ومقا ييس الدم :

وجد أن درجة الحمو ضة لسا ئل الكرش على اوقا ت مختلفة لاتوجد بينها اختلا فا ت معنو ية . كما أن نيتروجين أمونيا سائل الكرش والأحماض الدهنية الطيارة كا ن غير مختلف معنويا بين المجا ميع التجريبية المغذاه على القش المعا مل ببكتير يا لكنها تختلف عن الكنترول . ووجد ان مستوى البروتين الكلى واليوريا والكرياتينين لبلازما الدم لا يختلف معنويا بين المجا ميع التجريبية المغذاه على القش المعا مل ببكتير يا لكنها تختلف عن الكنترول .

مما سبق يمكن استنتاج انه يمكن استخدام بكتيريا Ruminococcus albus and Clostridium cellulovorans لمعاملة قش الأرز حيث أن المعا ملا ت البكتيرية قد نجحت فى زيادة نسبة البروتين الخام وزيادة قيم معاملات الهضم المعمليةو رفع القيمة الغذائية (TDN) لقش الأرز من 52.30 ٪ الى 70.08 ٪ مع خفض نسبة الألياف الخام مما يمكن من الأستفادة من قش الأرز بعد تحسين قيمته الغذائيةبالمعاملة البكتيرية فى تغذية الأغنام .