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Nutritive Improvement of Some Low Quality Roughages for Ruminants. I. Effect of Different Microbial and Chemical Treatments on the Quality of Sugar Cane Bagasse*

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TWO experiments were carried out to improve the nutritive value of sugar cane bagasse. In the 1st experiment, sugar cane bagasse was fermented with 4 different micro-organisms, namely **Trichoderma viride** 253 M-16, **Basidomycetes sp.** I, II and **Gliocladium sp.** Q 230. In the second one, sugar cane bagasse was fermented with **Trichoderma viride** (T.V.) fungi or treated with NaOH or sodium hypochlorite only. All microbial treatments used were greatly increased CP and ash and decreased CF, NFE, NDF, ADF, ADL and hemicellulose contents of bagasse. Values of IVDMD and **In Situ** DM disappearance increased ($P < 0.01$) with microbial treatments. A modification used during experiment II caused a decrease in ash content of T.V. fermented bagasse. Both NaOH and sodium hypochlorite treatments decreased CP, hemicellulose and ADL and increased ADF and cellulose contents of bagasse. Either two chemical treatments used or T.V. treatments increased significantly IVDMD values and **In Situ** DM disappearance at different incubation periods (4, 8, 12, 14 and 48 hr.). Highest improvement in the nutritive value of bagasse was obtained with the T.V. treatment.

It was concluded that fungal fermented bagasse or chemically treated bagasse can successfully be used in rations for ruminants. A combination of NaOH or sodium hypochlorite plus urea supplement would be beneficial to the nutritive value of such products.

The existing shortage of animal feeds in Egypt necessitates that intense research efforts should be directed towards exploring the possibility of using new-non-conventional sources or agricultural by-products as animal feeds and improving their nutritive values.

Sugar cane bagasse is one of these agricultural residues found in Egypt and surplus amounts of which are produced annually. Bagasse are highly lignified material and consequently of low digestibility and are not palatable. Many workers showed

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that nutrients digestibility and feed intakes of poor quality roughages or agricultural residues can greatly be improved by alkali treatments (Donefer, 1968) Abou-El-Hassan *et al.*, 1971, Chandra and Jackson, 1971, Singh and Jackson, 1971 and 1975, Braman and Abe, 1977, Jackson, 1977 and 1978 Orskov *et al.*, 1978 and 1980, Soliman *et al.*, 1984 and Abou-Raya *et al.*, 1984). Great quantities of digestible plant residues can be converted into food or feed by usage of different microorganisms or techniques (El-Hag, 1983 and Zadrazi, 1983).

The aim of the present work was to study the possibility of utilizing the crude sugar-cane bagasse as a new roughage source for ruminants and to try to improve its nutritive value by microbiological or chemical treatments.

Material and Methods

Two experiments were carried out in the present study. In the first experiment, a sample of the crude bagasse (untreated) and 4 samples of bagasse fermented with 4 different fungus namely; *Basidomycetes, sp.* I (BI), *Basidomycetes sp.* II (BII), *Gliocladium sp.* Q 230 (Q 230) and *Trichoderma viride* 253 M-16 (T.V.) were used. In experiment II, T.V. treated bagasse was used. Other two chemical treatments which were sodium hypochloride and NaOH treatments were compared with untreated bagasse and T.V. treated bagasse.

Gross chemical analysis along with Van Soest analysis were carried out for all samples used.

The nutritive value for each of the different samples was determined in terms of *In Vitro* dry matter disappearance (IVDMD).

In the second experiment, *In Situ* disappearance of dry matter in the rumen of the sheep was determined for untreated, sodium hypochlorite, NaOH and T.V. treated bagasse samples at different incubation periods using dacron bag technique.

Fungal Strains

The *Trichoderma viride* 253 M-16 employed is a mutant of the local strain *T. viride* 253 which was isolated from crude bagasse and identified through the Northern Regional Research

Laboratory, Peoria III 61604, U.S.A. *Basidomycetes sp.* I and II were kindly provided from the Botany Dept., Faculty of Science, El-Zagazig University as Cellulose decomposers. *Gliocladium sp.* Q 230 was isolated from crude bagasse and identified through the National Research Centre, Dokki, Egypt.

Maintenance of cultures

All cultures were maintained on Dox's agar medium. Slants were incubated at 30°C for 7 days and kept at 5°C.

Treatment of bagasse

Crude sugar-cane bagasse collected from Abou-Korkas Company from sugar manufacture was air dried and ground to 60 mesh size. The sodium hydroxide treated bagasse was prepared by steeping the ground bagasse with 2% NaOH (Solid : liquid ratio 1 : 10) for 10 hr., washed and then dried. The sodium hypochlorite treated bagasse was prepared by treatment of the bagasse with 0.7 g CL/1 g bagasse at pH 7 with glacial acetic acid for 20 hr at room temperature using the solid : liquid ratio 1 : 20 (Nadia *et al.*, 1984).

Fermentation

A solution of nutrients was used that contained (g/1) $(NH_4)_2SO_4$, 2.4 ; Calcium citrate, 1.0 ; KH_2PO_4, 2.0 ; glucose, 0.5 and peptone, 0.1. This formulation was found conducive for SCP production by the rested fungus (Farid *et al.*, 1984). 50 g of sodium hydroxide treated bagasse was incorporated into the nylon bags and mixed thoroughly with 60 ml. Portions of the nutritive solution (containing the balance equivalent to 2.5L) under unaseptic conditions. The content of each bag was then inoculated with 5% fungal inoculum, closed and incubated at room temperature for 30 days. After the incubation period the content of the bags were dried and used for the different determinations.

In experiment II calcium citrate was not added to the fermentation medium and the fermentation broth was washed before the chemical determinations.

In Vitro dry matter disappearance

In Vitro DM disappearance (IVDMD) of different samples was determined according to Norris *et al*, (1976). Rumen liquor was collected from Rahmani sheep using a stomach tube. The rams were maintained on all berseem hay diet (120% maintenance level).

Chemical analysis

Dry matter (DM), crude fiber (CF), ether extract (EE), crude protein (CP) and ash were determined according to A.O.A.C. (1970) procedures. Nitrogen free extract was obtained by difference. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970) procedures.

Determination of In Situ DM Disappearance

Dacron bag technique (Mehrez and Orskov, 1977) was used for determination of *In Situ* DM disappearance in the rumen of sheep at different incubation intervals (4, 8, 12, 24 and 48 hr).

Animals and their rations

Three mature male Ossimi sheep (of about 60 kg live weight and 3 years of age) fitted with rumen cannula were used. The animals were maintained on a basal diet of berseem hay given at a rate of 2.5% of body weight/head/day, in two equal meals mainly at 08.00 and 17.00. They were given access to water twice daily.

Dacron bags

Dacron bags of about 17 × 9 cm size were prepared using nylon thread in sowing. The material used in preparing the bags contained not less than 1700 holes/cm^2.

About 3 g of the ground samples (about 0.5 cm length) were weighed in each bag which was tied with nylon string, the other end of the string was tied to the top cover of the cannula through a wire hook fitted to it. The required number of bags were incubated in the rumen of sheep (not more than 5 bags) for the required period of incubation. Each sample was weighed in 3 bags for each incubation period and incubated in 3 sheep (triplicates). After the required period of incubation, the bags were removed from the sheep and washed thoroughly in running tap water. They were then dried using an oven at 60° for 48 hr. Disappearance of DM was calculated as the difference in weight of the sample before and after incubation.

Statistical analysis

Data concerning IVDMD and *In Situ* DM disappearance were statistically analyzed according to Snedecor and Cochran (1967), one or two-way classification followed by Duncan's multiple-range test to examine the significancy between means were used.

Results and Discussion

First Experiment

Data for chemical composition of untreated sugar-cane bagasse (control) and the fermented bagasse with different microorganisms are presented in Table 1. Results of the effect of different fungal treatments on the crude fibre fractions and nutritive value of sugar cane bagasses expressed as IVDMD are presented in Table 2.

Table (1) : Chemical analysis of bagasse untreated or treated with different microbial treatments.

Sample	Analysis, % DM					
	DM	CP	CF	EE	Ash	NFE
Control	93.1	1.7	45.2	2.4	4.7	46.0
Bas. I	93.9	15.9	32.1	2.9	19.5	29.6
Bas. II	94.4	17.9	32.6	1.8	19.5	28.2
T.V.	94.5	20.3	32.4	2.5	19.8	25.0
Q 230	94.8	21.1	29.2	1.8	20.7	27.2

The results showed clearly that fungal treatments affected the chemical composition of sugar-cane bagasse remarkably. The crude protein content increased and CF and NFE decreased. Zadrazil (1982) reported that a low protein content in plant residues can be increased after fermentation with different species of fungi.

It could be noticed from values given for different treatments that sugar-cane bagasse treated with different fungal treatments

had much higher ash content than untreated bagasse (4 : 1). The high ash content of fungal treatments is partially due to the salt added to the media during the treatment making.

Table (2) : Van Soest analysis and IVDMD of bagasse untreated or treated with different microbial treatments .

Sample	Crude fiber fraction, % DM					IVDMD	SE ±
	NDF	ADF	ADL	Hemice-llulose	Cellulose		
Control	84.5	57.7	18.5	26.8	39.2	^{A}21.5	1.6
Bas. I	46.6	40.6	13.6	6.0	27.0	^{B}48.8	2.0
Bas. II	44.3	38.9	11.0	5.4	27.9	^{B}48.5	1.4
T.V.	47.0	40.4	12.3	6.6	28.1	^{B}51.3	1.7
Q 230	49.8	43.1	16.7	6.7	26.4	^{B}52.9	1.0

A,B Means with different superscripts are significant ($P < 0.01$) different.

SE = Standard Error of the means.

Ether extract content of untreated sugar-cane bagasse or treated with different treatments were nearly the same.

The present results for chemical composition of untreated bagasse agreed well with those reported by Abou-Raya (1967) and Ahmed (1984).

Comparison of chemical composition of the different fungal treatments showed clearly that CF, EE, NFE and ash contents for the four fungal treatments were very close. However CP content of T.V. treatment was nearly similar to that of Q 230 treatment and the both were higher than that of the other two treatments.

Fungal treatments decreased NDF, ADF and ADL contents for sugar-cane bagasse. Values of NDF, ADF and ADL contents for the different fungal treatments were very close.

Fungal treatments of sugar cane bagasse greatly decreased hemicellulose and cellulose contents. Values obtained for the different fungal treatment were very close.

Fungal treatments increased significantly ($P < 0.01$) the nutritive value expressed as IVDMD for sugar cane bagasses from 21.5 to 52.9%. The improvement in the IVDMD values may have been related to the decrease in CF content and its fractions (Van Soest, 1967 ; Van Soest and Jones, 1968 ; Jackson, 1977 and Horn *et al.*, 1979) and the increased CP content with fungal treatments (Kempton and Leng, 1979). Moreover, the high soluble ash content with fungal treatments may explain the increase of IVDMD values (Shoukry, 1982).

The values obtained for IVDMD of untreated bagasse were lower than that reported by Ahmed (1983) (21.5 VS. 32.5%). This was possibly because the bagasse used in this study had lower CF content and its fractions than those in the present study.

Second Experiment

The results of experiment I showed that T.V. treatment gave the second IVDMD value compared with the other treatments and its ash content was lower than that of the treatment recorded highest IVDMD value. It was decided therefore, to select that treatment (T.V.) with a modification to reduce ash content of the products.

During this experiment, other two methods were used as chemical treatments, which were sodium hypochlorite and NaOH to compare its effect with T.V. treatment on the chemical composition and nutritive value of sugar cane bagasse. Chemical analysis and nutritional evaluation of the 4 treatments were carried out.

Results concerning chemical composition of sugar-cane bagasse untreated or treated with different treatments are presented in Table 3.

The results showed that sodium hypochlorite and NaOH treatments decreased CP and EE contents and increased ash content of bagasse. Sodium hypochlorite treatment decreased CF and increased NFE contents, while NaOH treatment increased CF and decreased NFE of bagasse.

Table (3) : Chemical composition of bagasse untreated or treated with different treatments .

Treatment	Composition, % D M					
	DM	CP	CF	EE	Ash	NFE
Untreated bagasse (control)	92.5	1.5	50.0	1.8	3.0	43.7
Sodium hypochlorite treated bagasse	91.1	1.1	46.4	1.5	3.4	47.6
NaOH treated bagasse	94.7	1.1	59.6	1.6	3.1	34.6
T.V. treated bagasse	90.6	11.8	54.6	2.1	8.1	23.4

Fungal treatments increased CP, CF, EE and ash and decreased NFE contents of bagasse.

The higher NFE content recorded for sodium hypochlorite treated bagasse compared with that of other two treatments may have been related to the great amount of soluble nutrients lost during washing step which was used in the two treatments (NaOH and T.V. treatments) and was not used in sodium hypochlorite treatment in the present study. Similar results have been reported by Abou-Raya (1967), who reported that treated bagasse with $Ca(OH)_2$ decreased its CF and NFE contents and increased ash content.

Many workers reported that treating poor quality roughages with NaOH or $Ca(OH)_2$ by using Soaking method increased CF and decreased NFE contents (El-Talty, 1973; Jackson, 1977 ; Salem, 1980 and Abou-Raya *et al.*, 1984). The cause of decreasing NFE content and increasing CF content of alkali treated poor quality roughages appeared to be mainly due to the losses of soluble nutrients.

Fractionation of the cell wall constituents of baggase untreated or treated with different treatments (Table 4) showed that either sodium hypochlorite or T.V. treatments decreased NDF content, while NaOH treatment increased NDF content of bagasse. Almost all treatments increased ADF and cellulose and decreased hemicellulose contents of bagasse. Both sodium hypochlorite and NaOH treatments decreased ADL content of bagasse. ADL content of T.V. treated bagasse was similar to that of untreated bagasse.

Table (4): Crude fiber fraction and IVDMD of bagasse untreated or treated with different treatments.

Treatment	Crude fiber fraction, % DM						
	NDF	ADF	ADL	Hemicellulose	Cellulose	IVDMD %	SE
Untreated bagasse (control)	90.0	63.2	16.9	26.8	46.3	^{A}22.1	± 1.3
Sodium hypochlorite bagasse	76.1	68.0	5.1	8.1	62.9	^{A}23.9	± 0.6
NaOH treated bagasse	94.3	70.4	12.4	23.9	58.0	^{B}35.6	± 0.8
T.V. treated bagasse	79.6	74.1	17.0	5.5	57.1	^{C}45.5	± 1.8

A,B ,C Means with different superscripts are significantly ($P < 0.01$) different .

SE = Standard error of the means.

Crude fibre fraction values obtained in experiment II for T. V. treatment were greater than those recorded for the same treatment in experiment I. This was possibly because the T. V. treated bagasse used in experiment II had higher CF and lower ash and CP contents than that used in experiment I.

Comparison of nutritive values in terms of IVDMD of the different treatments (Table 4) showed that highest value was recorded for T. V. treatment followed by that of NaOH treatment ($P < 0.01$). Values recorded for sodium hypochlorite treated bagasse were slightly greater than that recorded for untreated bagasse ($P < 0.05$). The superiority of T. V. treatment may have been related to the higher CP content of this treatment than the other treatments (Kempton and Leng, 1979). The results obtained agreed well with those reported by El-Torky (1979), who found that the IVDMD values of bagasse increased as the level of NaOH used increased.

Detailed results and overall means of *In Situ* DM disappearance of sugar-cane bagasse untreated or treated with different treatments at different incubation periods in the rumen of sheep are presented in Table 5. The results obtained showed that DM disappearance of sugar-cane bagasse untreated or treated with different treatments, increased significantly ($P < 0.01$) as the period of incubation increased from 4 to 48 hr.

Rate of DM disappearance was greater for T.V. treatment than the control (untreated bagasse) and the two chemical treat-

ments during the first 12 hr. From 12 to 48 hr rate of disappearance was almost similar for T. V. and the two chemical treatments and was much greater than for control. No significant differences were observed among the three treatments and there were significant differences ($P < 0.01$) between these treatments and the control.

The results obtained for *In Situ* DM degradability (Table 5) for almost all treatments agreed well with those recorded for IVDMD except for sodium hypochlorite treatment. In this treatment, values recorded for IVDMD were lower than that recorded for *In Situ* DM disappearance. This may have been related to the toxic effect of chlorine ions on the rumen microorganisms which

Table (5) : In Situ DM disappearance (%) of untreated or treated bagasse with different treatements at different incubation periods.

Treatments	Incubation period, hr.					Overall mean
	4	8	12	24	48	
Untreated bagasse	6.0	10.3	10.5	18.8	27.1	14.5 ^{A}
SE	±0.0	±0.0	±0.3	±0.8	±0.6	
Sodium hypochlorite treated bagasse	6.9	13.8	28.3	42.5	56.3	29.6 ^{B}
SE	±0.3	±0.2	±1.0	±1.0	±0.6	
NaOH treated bagasse	7.7	12.7	25.4	46.1	60.7	30.5 ^{B}
SE	±0.7	±0.6	±0.4	±1.2	±3.5	
T.V. treated bagasse	24.1	28.3	33.2	44.7	57.0	37.5 ^{B}
SE	±0.2	±1.1	±1.0	±1.4	±2.4	

^{A,B} Means with different superscripts are significantly ($P<0.01$) different.
SE = Standard error of the means.

appeared in the *In Vitro* technique and did not appear used dacron bag technique. This result emphasized the importance of washing materials treated with sodium hypochlorite before use it to remove the toxic effect of chloride ions.

The results obtained showed that *In Vitro* and *In Situ* DM disappearance of bagasse increased with NaOH, Sodium hypochlorite or fungal treatments. Similar results have been reported with poor quality roughages treated with NaOH or $Ca(OH)_2$ (El-Ayek, 1981; El-Serafy *et al.*, 1983; Soliman *et al.*, 1984 and Abou-Raya *et al.*, 1984). Jackson (1978) summarized the reason for the

increased nutrients disappearance with alkali treatment as : 1) solubilize hemicellulose, lignin and silica, 2) increases extent of cellulose and hemicellulose digestion and 3) increase rate of cellulose and hemicellulose digestion.

The present study suggested that chemical composition and nutritive value of sugar-cane bagasse can be improved by fungal treatment or chemical treatment. Work in progress to explore the extent to which treated bagasse can be used in place of common roughage sources in rations for ruminants. The effect of sprayed NaOH or sodium hypochlorite treated bagasse with urea solution on its nutritive value is needed to examine the effect of added urea on the basis of the equivalent nitrogen content of the product from T.V. treatment.

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زيادة القيمة الغذائية لبعض مواد العلف الخشنة الفقيرة في علائق المجترات :

١ ـ تاثير المعاملات الميكروبية والكيماوية المختلفة على القيمة الغذائية لمصاصة قصب السكر

محسن محمود شكرى ، فاروق هميسه ، سوسن منصور أحمد ، عبد المنعم الرفاعى ، حاتم محمد على وظباء محمد زكى عبد المنجلى .

معمل تغذية الحيوان والدواجن ومعمل كيمياء الكائنات الدقيقة ـ المركز القومى للبحوث ـ القاهرة .

تضمنت هذه الدراسة اجراء تجربتين ، فى الاولى تم معاملة مصاصة قصب السكر بأربع انواع مختلفة من الميكروبات (فطريات) وفى التجربة الثانية اختيرت احدى هذه المعاملات الفطرية وهى المعاملة بفطر التريكودرما فاردى وقورنت بالمعاملة الكيماوية اما بأيدروكسيد الصوديوم أو هيبوكلوريت الصوديوم ، وفى كل من التجربتين تم دراسة تاثير هذه المعاملات المختلفة على القيمة الغذائية لمصاصة قصب السكر .

وتشير النتائج الى ان جميع المعاملات الميكروبية المستخدمة ادت الى زيادة محتوى مصاصة قصب السكر من البروتين الخام والرماد الخام كما ادت الى خفض محتوى المصاصة من الالياف الخام ومكوناته من ADF, NDF ADL والسليولوز والهيمسليولوز وقد ادت جميع المعاملات الميكروبية الى زيادة معدل اختفاء المادة الجافة فى كرش الاغنام المقدرة بطريقتين الكرش الصناعى In Vitro والاكياس الداكرون In Situ نى التجربة الثانية امكن خفض محتوى مصاصة قصب السكر المعاملة بفطر التريكودرما فاردى من الرماد الخام . واوضحت النتائج ان معاملة المصاصة بكل من ايدروكسيد الصوديوم أو هيبوكلوريت الصوديوم ادت الى خفض محتواها من البروتين الخام والهيمسليولوز واللجنين بينما ادت الى زيادة محتواها من السيولوز و ADF

تشير النتائج الى ان معاملة مصاصة قصب السكر بفطر التريكودرما فاردى او ايدروكسيد الصوديوم أو هيبوكلوريت الصوديوم أدت الى زيادةمعدل اختفاء المادة الجافة المقدرة In Situ, In Vitro زيادة معنوية وكانت أكبر زيادة فى حالة استخدام المعاملة بالفطر . وبصفة عامة فأن النتائج تقترح امكانية زيادة القيمة الغذائية لمصاصة قصب السكرباستخدام المعاملة الميكروبية أو الكيماوية وامكانية استخدام المصاصة المعاملة بنجاح فى علائق المجترات كمادة علف خشنة .