Egyptian J. Nutrition and Feeds (2009) 12 (3) : 359-372

EVALUATION DIFFERENCES OF SOME RUMINAL BACTERIA BY *IN VITRO* DRY MATTER, CELLULOSE AND HEMICELLULOSE DISAPPEARANCE RATE AND EXTENT OF BAGASSE

H.M. Gado and Etab R.I. Abd El-Galil

Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

(Received 12/5/2009, Accepted 29/9/2009)

SUMMARY

The objectives of this paper are to evaluate the differences among five cellulolytic bacteria isolated from the rumen regarding *in vitro* dry matter, cellulose and hemicellulose disappearance. Cellulolytic bacteria such as *Cellulomonas cellulasea*, *Acetobacter xylinum*, *Thermonospora fusca*, *Ruminococcus albus* and *Bacillus sp.* had been isolated from cow, sheep, buffalo and camel. Sugarcane bagasse was incubated with each strain for 48 hrs incubation (extent) and the regression coefficient of *in vitro* was rate of disappearance per hour. The increase recorded of *in vitro* dry matter disappearance values. Five strains isolated from sheep were more effective than that isolated from buffalo and cow. The results revealed that *Cellulomonas* and *Ruminococcus* isolated from camel showed the highest ($p<0.05$) value of *in vitro* cellulose disappearance of bagasse (65% and 62%). While *Bacillus* isolated from sheep showed the highest ($p<0.05$) value of *in vitro* cellulose and hemicellulose digestion (53% and 47%, respectively). *Thermonospora* and *Acetobacter* isolated from cow showed higher of *in vitro* cellulose digestion (56% and 46%) more than those isolated from camel, sheep and buffalo. The results recorded that *Thermonospora* isolated from cow had the highest value of *in vitro* hemicellulose disappearance (52%), while *Cellulomonas* isolated from buffalo showed the highest value (64%). *Acetobacter* and *Ruminococcus* isolated from camel showed the highest value of hemicellulose (44% and 62%). It was concluded that the five strains isolated from four ruminant animals significant differences on *in vitro* dry matter, cellulose and hemicellulose disappearance of sugarcane bagasse with the same strain.

Keywords: bagasse, cellulolytic bacteria, in vitro, dry matter, cellulose, hemicellulose.

INTRODUCTION

Agricultural residues, such as wheat straw, rice straw and bagasse, contain considerable quantities of cellulose and hemicellulose that could be an energy sources in ruminants feed (Detroy *et al.*, 1980). Williams and Withers, (1985) demonstrated that the specific activities of hemicellulose-degrading polysaccharidases and glycosidases were influenced by both the rate and stage of growth. In batch culture, the specific activities increased throughout the growth cycle to reach maximum levels in the late exponential and stationary growth phases. Cultures the activities were growth-rate dependent, and although activity was maintained over a wide range of growth rates, the activities were lower in more rapidly growing cells. An enzyme activity in *Ruminococcus Flavefaciens*, another

rumen polysaccharolytic strain, varies with the growth rate and stage of growth (Pettipher and Latham, 1979). Untreated lignocelluloses can only be decomposed under special conditions and with a few microbial species (Crawford *et al.*, 2004). Gill *et al.*, (1995) recorded that a correlation of 0.993 was found between relative rate of digestible cellulose disappearance *in vitro* and digestible dry matter intake of cows consuming high dry matter legume-grass silage. It was concluded that relative rate of *in vitro* cellulose digestion is a promising method for estimating digestible dry matter intake of forages. Abd El-Galil (2000) showed that enzymatic treatment (cellulase enzymes) of bagasse improved its chemical composition, cell wall constituents, *in vitro* dry matter and organic matter disappearance.

Fisher *et al.*, (1989) concluded that understanding the dynamics or kinetics of digestion and passage may suggest ways to manipulate forages to improve the diet, and consequently the production, of domesticated ruminants. The methodology of fitting exponential curves was tested using simulated cell-wall-disappearance curves with both an exponential and a gamma-distributed exponential driving function and normal-random variation superimposed. Nonlinear regression gave more precise estimates of the true rates of digestion and lag times than linearization. Mixtures of high with low digestible forages could improve microbial colonization of cell walls (Ortiz *et al.*, 2001). Abd El-Galil (2006) found that *Cellulomonas cellulasea, Acetobacter xylinum, Thermonospora fusca, Ruminococcus albus, and Bacillus sp.* originally isolated from the rumen of goats, can grow under anaerobic conditions. These strains were different in the percent and type of cellulase enzymes, 28.57% in *Ruminococcus albus*, 43.75% in *Thermonospora fusca*, 30.30% in *Acetobacter xylinum*, 25.92% in *Bacillus sp* and 33.33% in *Cellulomonas cellulasea*. There are major cellulolytic bacteria within the rumen. One of the reasons for its predominance isolation is its ability to readily degrade various forms of crystalline cellulose, hemicellulose and lignocelluloses materials.

The present study was carried out to main objective comparative of some cellulolytic bacteria (*Cellulomonas, Acetobacter, Thermonospora, Ruminococcus* and *Bacillus*) isolated from some species of ruminant animals (cow, buffalo, camel and sheep) on *in vitro* dry matter, cellulose and hemicellulose disappearance rate and extent of sugarcane bagasse as agricultural by- products.

MATERIALS AND METHODS

Preparation of bacterial cultures:-

Four strains and one species of cellulolytic bacteria were isolated from rumen fluid of ruminant animals and were grown as pure cultural. Fluids collected immediately from different animals after animal slaughter in the slaughter house of Basatein. Four strains and one species of cellulolytic bacteria from cow, buffalo, camel and sheep were prepared to *in vitro* dry matter, cellulose and hemicellulose disappearance .The separated stains are:

1- *Cellulomonas cellulasea* 2- *Acetobacter xylinum*
3- *Thermonospora fusca* 4- *Ruminococcus albus* 5- *Bacillus sp.*

Pure culture media:

Isolation of species was used the streak-plate or the pour-plate method. The pour-plate technique for 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 were support 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, it is allowed to solidify, and the plates are incubated, Bacteria suspended in the milted agar are trapped in the agar as it solidifies. 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 dilutions, in which bacteria are physically separated from each other, discrete colonies are observed.

Invitro dry matter, cellulose and hemicellulose disappearance:

The *in vitro* dry matter, cellulose and hemicellulose disappearance rate and extent was determined according to method described by Terry *et al.*, (1969). Two tubes, as a replicate of each sample, were used at different incubation times (2, 4, 6, 24, 48 hrs.).

Proximate analysis:-

The proximate analysis of sugarcane bagasse was 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 sugarcane bagasse was 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* dry matter disappearance, *in vitro* hemicellulose disappearance, *in vitro* cellulose disappearance and Regression coefficient (rate) were statistically analyzed according to statistical analysis system 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:

$$Y_{ij} = \mu + S_i + Bk + a_{ij}k$$

Where: Y ij = the observation of the model, μ = General mean common element to all observation, Si = the effect of the strains (i = 1... 5), BK= the effect of the animals (K = 1....4), a ij = The effect of error.

RESULTS AND DISCUSSION

Chemical composition and cell wall constituents of sugarcane bagasse

Chemical composition and cell wall constituents of sugarcane bagasse are presented in Table (1). The present data indicated that the value of crude protein (CP) was 1.74 %, but the value of crude fiber (CF) was 41.9 %. The value of ether extract, NFE and Ash were 2.0%, 53.29% and 1.08%, respectively. The data indicated that the value of NDF was 63.4

%, while the values of ADF, ADL, hemicellulose and cellulose were 43.6 %, 14.0%, 19.8% and 29.6%, respectively. These data confirmed the increases of cell wall constituents in sugarcane bagasse. This raw material needs to degrade of crude fiber and cell wall constituents with cellulase enzymes secreted by cellulolytic bacteria to convert feedstuff to feed ruminant animals (Tiwari *et al.*,2007). Researchers had developed a new feeding standard using high fiber diets, particularly based on sugarcane bagasse as a large tropical crop that offers great challenge for ruminal nutrition (Galina *et al.*, 2003). Sugarcane bagasse components need wide ruminal microbial populations to favor fermentation (Galindo *et al.*, 1993).

Table (1): Chemical composition and cell wall constituents of untreated sugarcane bagasse (% on DM basis)

Item	DM	OM	CP	CF	EE	NFE	Ash	NDF	ADF	ADL	Hemicellulose	Cellulose
Sugarcane bagasse	92.05	98.90	1.74	41.90	2.00	53.29	1.08	63.40	43.60	14.00	19.80	29.60

The extent of in vitro dry matter disappearance

Data in Table (2) exhibited the effects of some cellulolytic bacteria from many species of ruminant animals on *in vitro* dry matter disappearance (extent) of sugarcane bagasse.

The highest significant ($p<0.05$) value was found in *Cellulomonas cellulasea* isolate from sheep (64.8 %) while the lowest significant value was found in *Cellulomonas cellulasea* isolate from cow (51.7%). Finally the data showed that the highest values found by *Cellulomonas cellulasea* which isolate from sheep then camel and buffalo (60% and 59.6%) but the lowest value found by *Cellulomonas cellulasea* isolate from cow. Generally, the values increased *in vitro* dry matter disappearance with *Cellulomonas cellulasea* which isolate from sheep most effective on *in vitro* dry matter disappearance.

Table (2): The extent of *in vitro* dry matter disappearance of bagasse with some cellulolytic bacteria from ruminant animals (% on DM basis)

Item	Cow	Buffalo	Camel	Sheep
Cellulomonas cellulasea	51.5	59.6	60	64.8
Bacillus sp	52.4	60.4	59.2	63.2
Acetobacter xylinum	58.6	59.4	60.2	61.8
Thermonospora fusca	57	51.3	56.4	61.7
Ruminococcus albus	59	60.8	61.6	62.7

In Table (2), the highest value was found in *Bacillus sp.* isolate from sheep (63.2%) while the lowest value was found in *Bacillus sp.* isolate from cow (52.4%). Generally, the value increased *in vitro* dry matter disappearance with *Bacillus sp.* isolate from sheep was more effective than isolate from cow, buffalo and camel. This data indicated that the value

of *in vitro* dry matter disappearance of bagasse with *Bacillus sp.* was the high effect when isolate from sheep.

Data in Table (2) are cleared that *Acetobacter xylinum* isolate from sheep recorded the highest ($p<0.05$) value (61.8%) then camel (60.2%) after that buffalo (59.4%) while the lowest value found in *Acetobacter xylinum* isolate from cow (58.6%). Generally, the value of *in vitro* dry matter disappearance with *Acetobacter xylinum* isolate from sheep was more effective than isolate from buffalo, camel and cow.

In Table (2), the highest value found in *Thermonospora fusca* isolate from sheep (61.7%) while the lowest value found in *Thermonospora fusca* isolate from buffalo (51.3%). Generally, the value of *in vitro* dry matter disappearance with *Acetobacter xylinum* isolate from sheep was more effective than isolate from buffalo, camel and cow.

In Table (2), the highest value found in *Ruminococcus albus* isolate from sheep (62.7%) then camel (61.6%). While the lowest value found in *Ruminococcus albus* isolate from cow (59%). Generally, the value of *in vitro* dry matter disappearance with *Ruminococcus albus* isolate from sheep and camel (62.7% and 61.6%) were more effective than isolate from buffalo and cow (60.8 % and 59%, respectively).

These results due to the strains of cellulolytic bacteria isolate from sheep were the most effective on *in vitro* dry matter disappearance of sugarcane bagasse which showed the highest values of *in vitro* dry matter disappearance because these active strains were secreted cellulase enzymes most effective on sugarcane bagasse. Abd El Galil (2006) she suggest that four strains and one species of cellulolytic bacteria isolate from goats were similar in containing cellulase enzymes. However, variation in molecular weight and present of cellulase presence suggest the specification of each strain in secreting special combination of these enzymes and consequently in breaking down special cellulasic or lignocellulosic band.

Tripathi and Yadar (1992) fermented wheat straw with the selected white – rot fungus *(Pleurotus ostreatus)*. They found that, the *in vitro* dry matter digestibility was increased (10.40 %) and degradation of lignin was attained in the optimized solid state fermentation. Kundu, (1994) *in vitro* DM, NDF and ADF digestibility were decreased due to fermentation with fungi. Rai and Mudgal (1984) reported an increase in IVDMD was observed from 67.99 % to 77.55 % for control and treated wheat straw (with *T. viride*), respectively. Raman and Naik (1993) cultivated *Pleurotus ostreatus* on dried chapped wheat straw and rice straw in ultra violet chamber for solid state fermentation. The cultivation technique was carried out at different levels of moisture (50, 80 and 100 %), using incubation temperature of 27, 28 C. They observed an increased *in vitro* dry matter digestibility and crude protein in all treatment. However, CP and *in vitro* dry matter digestibility were increased gradually with increasing incubation period for both straws. Improvement in nutritive value of wheat straw and rice straw was evident with species of fungus. Abdul Aziz *et al.*, (1994) fermented rice straw, bean straw and sugarcane bagasse with *Pleurotus ostreatus* and found that IVDMD and IVOMD of rice straw were significantly improved compared to raw material. Abdul – Aziz *et al.*, (1997) reported that, levels increased were 42.13 %, 31.45 % and 14.6 % for IVDMD, IVOMD and IVCFD, respectively when they treated rice straw by *Pleurotus ostreatus*. Moreover, Rai and Mudgal (1984) found that digestibility *in vitro* of DM, cell wall; ADF and hemicellulose were higher ($p < 0.01$) in treated straw with cellulase enzymes than untreated one.

In addition, an increase of 12 percentage units in the *in vitro* digestibility of wheat straw incubated with *Pleurotus florida* was reported by Zadrazil (1977). Abd El-Galil (2006) observed that very high rate of improvement was recorded of IVOMD treated bagasse with *Cellulomonas cellulasea*; *Acetobacter xylinum* and *Ruminococcus albus* (67.8%, 68.6% and 75.6%, respectively) compared untreated bagasse (48.9%). And the values of IVDMD bagasse treated with *Cellulomonas* and *Acetobacter* were improved to 80.1% and 81.6%, respectively compared untreated bagasse 68.8%. These results are in a good agreement with results obtained by Zadrazil and Brunnert, (1980), Shoukry *et al.*, (1985) and Abdul – Aziz *et al.*, (1994). Abd El-Galil (2000) observed that, addition of 15% cellulase enzyme to bagasse caused the highest values IVDMD and IVOMD while the untreated bagasse and the other treatments were the lowest values. She found that values of IVDM and IVOM disappearances for bagasse treated with cellulase enzymes recorded 59.55% and 66.2%, respectively.

Rate of in vitro dry matter disappearance

The regression coefficients (rate = unit / hour) of *in vitro* dry matter disappearance of bagasse on species of cellulolytic bacteria from different species of ruminant animals are showed in Table (3). The data showed that the regression coefficients between many species of ruminant animals which the rate of *Cellulomonas cellulasea* were the highest values in cow and camel (1.064 and 1.001) then in sheep (0.9409) while the lowest value was in buffalo (0.817). The rate of *Bacillus sp.* in cow and camel were the highest values (1.022 and 1.024) then in sheep (0.954) but the lowest value was in buffalo (0.827). The rate of *Acetobacter* in buffalo was the highest value (0.987) then in camel and sheep (0.976, 0.961) while the lowest value of the rate was in cow (0.9118). The rate of *Thermonospora* in buffalo was the highest value (1.061) then in cow and sheep (0.939 and 0.933) while in camel was the lowest value (0.902). The rate of *Ruminococcus* in camel was the highest values (1.013) then in sheep and buffalo (0.987 and 0.957) while in cow (0.889) was the lowest value. Generally, in camel found that the highest active strains on *in vitro* dry matter disappearance rate were *Cellulomonas, Bacillus and Ruminococcus* while in cow found that the highest active strains were *Cellulomonas, Bacillus* and *Acetobacter*. In buffalo found that the highest active strains on *in vitro* dry matter disappearance rate were *Thermonospora* and *Acetobacter*. In sheep showed that the most active strains on *in vitro* dry matter disappearance rate were *Cellulomonas, Bacillus, Acetobacter, Thermonospora* and *Ruminococcus* which in all strains found the same values approximately around 0.9 recorded.

Table (3): Rate of *in vitro* dry matter disappearance of bagasse with some cellulolytic bacteria from ruminant animals (rate = unit / hour)

Item	Cow	Buffalo	Camel	Sheep
Cellulomonas cellulasea	1.064489	0.817767	1.001132	0.940930
Bacillus sp	1.022366	0.827503	1.024762	0.954352
Acetobacter xylinum	0.911837	0.987303	0.976797	0.961522
Thermonospora fusca	0.939738	1.061019	0.902145	0.933110
Ruminococcus albus	0.889975	0.957444	1.013741	0.987364

The extent of in vitro cellulose disappearance

Data in Table (4) exhibited the effects of some cellulolytic bacteria from many

species of ruminant animals on *in vitro* cellulose disappearance (extent) of sugarcane bagasse. The highest significant ($p<0.05$) value was in *Cellulomonas cellulasea* isolated from camel (65%), while the lowest significant value was in *Cellulomonas* isolated from cow (58%). Finally, the highest values found with *Cellulomonas* which isolated from camel then buffalo and sheep while the lowest value found was from cow.

In Table (4), the highest value was in *Bacillus* isolated from sheep (53%) then camel and buffalo (49% and 48%) while the lowest value was in *Bacillus sp.* isolated from cow (46%). Generally, the value of *in vitro* cellulose disappearance with *Bacillus sp.* isolated from sheep showed more effect than that isolated from cow, buffalo and camel. This data indicated that the value of *in vitro* cellulose disappearance of bagasse by *Bacillus sp.* showed a slightly effect when isolated from cow, buffalo and camel.

Table (4): The extent of *in vitro* cellulose disappearance of bagasse with some cellulolytic bacteria from ruminant animals (% on DM basis)

Item	Cow	Buffalo	Camel	Sheep
Cellulomonas cellulasea	58	63	65	63
Bacillus sp	46	48	49	53
Acetobacter xylinum	46	44	43	42
Thermonospora fusca	56	49	52	51
Ruminococcus albus	57	54	62	57

Data in Table (4) illustrated that *Acetobacter xylinum* isolated from cow and buffalo recorded the highest values (46% and 44%, respectively) then camel and sheep (43% and 42%, respectively). Generally, the value of *in vitro* cellulose digestibility with *Acetobacter xylinum* isolated from cow was more effect than isolated from buffalo, camel and sheep. This data indicated that value of *in vitro* cellulose disappearance with *Acetobacter xylinum* which isolated from buffalo, camel and sheep have no significant differences.

In Table (4), the highest value ($p<0.05$) found in *Thermonospora fusca* isolated from cow (56%) then camel (52%) and sheep (51%) while the lowest value was appeared in *Thermonospora fusca* isolated from buffalo (49%). Generally, the value of *in vitro* cellulose disappearance with *Thermonospora* isolated from cow was more effective than isolated from camel, sheep and buffalo.

In Table (4), the highest value ($p<0.05$) was in *Ruminococcus albus* isolated from camel (62%) then cow and sheep (57% and 57%) while the lowest value was in *Ruminococcus albus* isolated from buffalo (54%). Generally, the value of *in vitro* cellulose disappearance with *Ruminococcus albus* isolated from camel was more effective than form sheep, buffalo and cow similarly as Pandey *et al.*, (2000). These results due to three strains (*Cellulomonas, Bacillus* and *Ruminococcus*) of cellulolytic bacteria isolated from sheep were most effective on *in vitro* cellulose disappearance of sugarcane bagasse while two strains (*Ruminococcus* and *Cellulomonas*) isolated from camel were most effective on *in vitro* cellulose disappearance. But two strains (*Thermonospora* and *Acetobacter*) isolated from cow were most effective on *in vitro* cellulose disappearance. So, the effects of *Cellulomonas cellulasea* isolated from camel showed the highest value of *in vitro* cellulose disappearance of sugarcane bagasse.

Rate of in vitro cellulose disappearance

The regression coefficients (rate = unit / hour) of *in vitro* cellulose disappearance of bagasse on cellulolytic bacteria species isolated from many ruminant animals are showed in Table (5). The data showed that the regression coefficients between many ruminant animals species which rate of *Cellulomonas cellulasea* was the highest value in sheep (1.048), then in camel and buffalo (0.979 and 0.974, respectively) while the lowest value was in cow (0.87). The rate of *Bacillus sp.* in cow showed the highest value (1.25), then in buffalo (1.039) and in camel (0.964) but the lowest value was in sheep (0.872). The rate of *Acetobacter* in cow was the highest value (1.32), than in sheep, buffalo and camel, the same values approximately (1.08, 1.04 and 1.01, respectively) were obtained. The rate of *Thermonospora* was the highest value (1.13) in buffalo then in cow (0.989) and gave the same values in camel and sheep approximately (0.958 and 0.951, respectively). The rate of *Ruminococcus* in sheep and buffalo showed the highest values (1.107 and 1.088, respectively) then in cow (0.992) while the lowest rate value was in camel (0.822). Generally, in sheep, the highest active strains were *Cellulomonas, Acetobacter* and *Ruminococcus* while in cow, the highest active strains were *Bacillus* and *Acetobacter.* In buffalo, the highest active strains were *Thermonospora, Acetobacter* and *Bacillus.* In camel, the highest active strains were *Cellulomonas, Acetobacter* and *Thermonospora.* For our knowledge, this is the first report discussing *in vitro* cellulose disappearance rate of sugarcane bagasse.

Table (5): Rate of *in vitro* cellulose disappearance of bagasse with some cellulolytic bacteria from ruminant animals (rate = unit / hour)

Item	Cow	Buffalo	Camel	Sheep
Cellulomonas cellulasea	0.870282	0.974659	0.979613	1.048185
Bacillus sp	1.256119	1.039991	0.964624	0.872121
Acetobacter xylinum	1.326858	1.047484	1.017751	1.083732
Thermonospora fusca	0.989630	1.131801	0.958716	0.951542
Ruminococcus albus	0.992104	1.088037	0.822287	1.107983

The extent of in vitro hemicellulose disappearance

Data in Table (6) exhibited the effects of some cellulolytic bacteria from many species of ruminant animals on *in vitro* hemicellulose disappearance (extent) of sugarcane bagasse. The highest significant value was in *Cellulomonas cellulasea* isolated from buffalo (64%), while the lowest significant value was in *Cellulomonas cellulasea* isolated from camel (61%). Generally, the value of *in vitro* hemicellulose disappearance with *Cellulomonas* which isolate from buffalo was more effective than isolate from sheep, camel and cow.

Table (6): The extent of *in vitro* hemicellulose disappearance (extent) of bagasse with some cellulolytic bacteria from ruminant animals (% on DM basis)

Item	Cow	Buffalo	Camel	Sheep
Cellulomonas cellulasea	62	64	61	63
Bacillus sp	44	45	46	47
Acetobacter xylinum	43	42	44	41
Thermonospora fusca	52	50	49	51
Ruminococcus albus	59	58	62	60

In Table (6), the highest ($p<0.05$) values found in *Bacillus sp.* isolate from sheep (47%) while the lowest values found in *Bacillus sp.* isolate from cow (44%). Generally, the

value of *in vitro* hemicellulose disappearance with Bacillus sp. isolate from sheep was more effective than isolate from cow, buffalo and camel.

Data in Table (6) are cleared the highest ($p<0.05$) values found in *Acetobacter xylinum* which isolated from camel (44%), while the lowest values found in *Acetobacter xylinum* isolate from sheep (41%). Generally, the value of *in vitro* hemicellulose disappearance with Acetobacter xylinum isolate from camel was more effective than isolate from buffalo, sheep and cow.

In Table (6), the highest ($p<0.05$) values found in *Thermonospora fusca* isolate from cow (52%) while the lowest values found in *Thermonospora fusca* isolate from camel (49%). Generally, the values of *in vitro* hemicellulose disappearance with *Acetobacter xylinum* isolate from cow and sheep (52 % and 51%) were more effective than isolate from buffalo and camel.

In Table (6), the highest ($p<0.05$) values found in *Ruminococcus albus* isolate from camel (62%), while the lowest values found in *Ruminococcus albus* isolate from buffalo (58%). Generally, the *in vitro* hemicellulose disappearance value with *Ruminococcus albus* isolate from camel was more effective than isolate from sheep, buffalo and cow.

These results due to two strains of cellulolytic bacteria isolate from sheep (*Cellulomonas* and *Bacillus*) were most effective on *in vitro* hemicellulose disappearance of sugarcane bagasse while two strains isolate from camel (*Ruminococcus* and *Acetobacter*) were most effective on *in vitro* hemicellulose disappearance. Two strains isolate from cow (*Thermonospora*) and buffalo (*Cellulomonas)* were most effective on *in vitro* hemicellulose disappearance,

Gill *et al.*, (1995) have shown that *in vitro* cell wall disappearance in forages is a first-order reaction in which the rate of disappearance is the product of proportionality constant and the concentration of digestible substrate. In their work dry matter intake was positively correlated with the proportionality constant. Smith *et al.*, (1972) reported significant correlation between the rate constant and percent lignin in the plant tissue of several grass and legume forages. Lignin – cellulose ratio and soluble dry matter were also correlated with the rate constant. Abd El Galil (2006) she suggests that four strains and one species of cellulolytic bacteria isolate from goats were similar in containing cellulase enzymes. However, variation in molecular weight and present of cellulase presence suggest the specification of each strain in secreting special combination of these enzymes and consequently in breaking down special cellulasic or lignocellulosic band.

Rate of in vitro hemicellulose disappearance

The regression coefficients (rate = unit / hour) of *in vitro* hemicellulose disappearance of bagasse on species of cellulolytic bacteria from many species of ruminant animals are showed in Table (7). The data showed that the regression coefficients between many species of ruminant animals which the value of *Cellulomonas cellulasea* was the highest in camel (1.035) then in sheep (0.959) while the lowest value was in cow (0.893). The value of *Bacillus sp.* in cow was the highest (1.345) then in sheep (1.058) but the same values were in buffalo and camel approximately (0.995 and 0.968). The value of *Acetobacter* in cow was the highest (1.511) then in camel and buffalo were the same values approximately (1.027 and 1.002, respectively) while the lowest value in sheep (0.946). The value of *Thermonospora* in cow was the highest value (1.157) then in camel (1.045) while in sheep

was the lowest values (0.911). The value of *Ruminococcus* in cow was the highest value (1.012) then in sheep and buffalo (0.983 and 0.982) were the same values approximately while in camel (0.907) was the lowest value. Generally, in camel found that the highest active strains of *in vitro* hemicellulose disappearance were *Cellulomonas, Acetobacter and Thermonospora* while in cow found that the highest active strains were *Bacillus, Acetobacter* and *Thermonospora*. In buffalo found that the highest active strains were *Bacillus, Acetobacter* and *Ruminococcus*. In sheep showed that the highest active strains were *Cellulomonas, Bacillus* and *Ruminococcus*.

Table (7): Rate of *in vitro* hemicellulose disappearance of bagasse with some cellulolytic bacteria from ruminant animals (rate = unit /hour)

Item	Cow	Buffalo	Camel	Sheep
Cellulomonas cellulasea	0.893408	0.904803	1.035211	0.959358
Bacillus sp	1.345204	0.995064	0.968954	1.058342
Acetobacter xylinum	1.511158	1.002174	1.027972	0.946663
Thermonospora fusca	1.157805	0.964704	1.045867	0.911131
Ruminococcus albus	1.012021	0.982229	0.907112	0.983824

Gado *et al.* (2009) concluded that *Cellulomonas and Bacillus* isolated from camel had the highest value of *in vitro* fermentation of cellulose of bagasse (44.17% and 30.5%) and followed by bacteria isolated from sheep (42.50% and 30.84%). *Thermonospora fusca* isolated from cow showed higher fermentation (32%) more than those isolated from camel, sheep and buffalo (30%, 29.83% and 29.33%, respectively). *Ruminococcus albus* isolated from camel (28.5%) had higher performance than those isolated from sheep, buffalo and cow (27.5%, 27.33% and 26.17%, respectively) ,while *Cellulomonas cellulasea* isolate from camel showed the highest mean of *in vitro* fermentation of cellulose (44.17%) of bagasse. It showed that five strains from four ruminant animals were significant differences on *in vitro* fermentation of cellulose of sugarcane bagasse in same strain.

CONCLUSION

The present data indicated that values of *in vitro* dry matter, cellulose and hemicellulose disappearance with all strains which isolate from cow, buffalo, camel and sheep were significant differences and all strains from sheep were the most active on *in vitro* dry matter disappearance of sugarcane bagasse. In sheep, the highest active strains on *in vitro* dry matter were all strains isolate from rumen of sheep while the highest active strains on *in vitro* hemicellulose disappearance were *Bacillus* and *Cellulomonas*. In cow, the highest active strains on *in vitro* hemicellulose was *Thermonospora*. In buffalo, the highest active strains on *in vitro* hemicellulose were *Cellulomonas* and *Acetobacter*. In camel, the highest active strains on *in vitro* dry matter were *Acetobacter* and *Ruminococcus* while the highest active strains on *in vitro* hemicellulose were *Cellulomonas, Acetobacter* and *Ruminococcus*.

These results due to one strain (*Bacillus*) of cellulolytic bacteria isolate from sheep were most effective on *in vitro* cellulose disappearance extent of sugarcane bagasse while two strains (*Ruminococcus* and *Cellulomonas*) isolate from camel were most effective on

in vitro cellulose disappearance. But two strains (*Thermonospora* and *Acetobacter*) isolate from cow was most effective on *in vitro* cellulose disappearance.

It was concluded that five strains from four ruminant animals were significant differences on *in vitro* dry matter, cellulose and hemicellulose disappearance of sugarcane bagasse of the same strain.

REFERENCES

A.O.A. C. (1990). Association of Official Analytical Chemists. Official methods of analysis (15th Ed.), Arlington, V.A.USA.

A.T.C. C. (1992). American Type Culture Collection Catalogue of Bacteria and Bacterophages (18th Ed.), USA.

Abd El-Galil, Etab. R.I. (2000). Nutritional factors affecting the performance of small ruminants. M.Sc. Thesis, Faculty of Agriculture. Ain-Shams University.pp.55-90.

Abd El-Galil, Etab. R.I. (2006). Effect of biological treatments on silage and feeding value of roughages in ruminants. Ph. D. Thesis, Faculty of Agriculture. Ain-Shams University. pp. 43-47

Abdul-Aziz, G.M.; A.M. Abdel-Gawad and M.S. Farghaly. (1994). Evaluation of untreated some poor quality roughages and it's spent. Egyptian J. Animal Prod. vol. 31: 191-201.

Abdul-Aziz, G.M.; Y.E. El-Talty and M.A. Ali. (1997). Biological treatments of rice straws in animal nutrition. Egyptian J. Nutrition and Feeds. Nov. special Issue. 225-234.

Crawford, A.C.; J.A. kricker; A.J. Anderson; N.R. Richardson and P. Mather. (2004). Structure and function of a cellulase gene in red claw crayfish cherax quadricarinatus. Gene. 340(2): 267-274.

Detroy, R.W.; L.A. Linden Felser; S.T. Julian and W.L. Orton. (1980). Saccharification of wheat straw cellulose by enzymatic hydrolysis following fermentative and chemical pretreatment. Biotechnology Bioengineering Symposium.10:135-148.

Duncan, D.B.(1955). Multiple rang and multiple F tests. Biometrics, 11:1.

Fisher; D.S., J.C. Burns and K.R. Pond.(1989). Kinetics of in vitro cell-wall disappearance and in vivo digestion. Agron. J. 81:25-33 .

Gado , H.M. ; Rania A.A. Younis and Etab R.I. Abd El-Galil. (2009). Molecular identification of some cellulolytic bacteria from different species of ruminant animals and *in vitro* fermentation of cellulose of sugarcane bagasse. Egypt. J. Genet. Cyto. 38:165-185.

Galina, M.A.F., Perez-Gil, F., Hummel, J.D., Ortiz, R.M.A., Orskov, E.R. (2003). Effect of slow intake urea supplementation on fatten- ing of steers feed sugar cane tops (Saccarum officinarum) and maize (Zea mays) with or without SIUS. Ruminal

fermentation, feed intake and digestibility. Livest. Prod. Sci. 83, 1-11.

Galindo, J., Stuart, R., Fundora, O., Regalado, E., Piedra, R., Delgado, D. (1993). Efecto del genotipo en la poblaci´n microbiana ruminal de toros que consumen residuos de centro de limpieza de ca˜a (Effect of genotype in ruminal microbial population in lambs fed a sugar cane factory by product). Rev. Cubana Cienc. Agrc. 27, 273-276.

Gill; S. S., H. R. Conrad and J. W. Hibbs .(1995).Relative Rate of in Vitro Cellulose Disappearance as a Possible Estimator of Digestible Dry Matter Intake . J. Anim. Sci., vol. 73: 3639-3648 .

Kundu, S.S. (1994). Composition changes and in-vitro digestibility of wheat straw fermented with sporulation fungal. Indian J. Dairy Sci. 47: 10-16.

Ortiz, R.M.A., Haenlein, G.F.W., Galina, M. (2001). Effects on feed intake and body weight gain when substituting maize with sugar cane in diets for Zebu steers complemented with slow release urea supplements. Int. J. Anim. Sci. 16, 239-245.

Pandey, A., Carlos R. Soccol, Poonam Nigam and Vanete T. Soccol (2000). Biotechnological potential of agro-industrial residues. I: sugarcane bagasse Bioresource Technology, 1: 69-80.

Pettipher, G.L. and M.J. Latham. (1979). Production of enzymes degrading plant cell walls and fermentation of cellobiose by Ruminococcus Flavefaciens in batch and continuous culture. J.Gen. Microbiol. 110: 29-39.

Rai, S.N. and V.D. Mudgal. (1984). Utilization of poor quality roughages: 2. Enzymatic treatment of wheat straw Asian. J. Dairy Res., 3(4): 193-200.

Raman, R. and D.G. Naik. (1993). Evaluation of biological fermentation of strains. Indian J. Animal Sci. 63(12): 1308-1309.

S.A.S. (1998). Statistical analysis system. User's Guide Inst., Inc.Cary, NC,USA.

Shoukry, M.M.; F.A. Hamissa ; M. Sawsan ; A.H. El-Refi ; H. M. Ali and Z. M. Z. Abdel-Motagally.(1985). Nutritive improvement of some low quality roughages for ruminants .1- Effect of different microbial and chemical treatments on quality of sugarcane bagasse. Egyptian J. Animal Production.25:329-336.

Smith, L.W.; H.K. Goering and C.H. Gordon. (1972). Relationships of forage composition with rates of cell wall digestion and indigestible contents. J. Dairy Sci. 55: 1140-1150.

Terry, R.A., J.M.A. Tilley, and G.E. Outen (1969). Effect of pH on cellulose digestion under in-vitro Conditions. J. Sci F. Agric., 20:317.

Tripathi, J.P. and J.S. Yadar. (1992). Optimization of solid substrate fermentation of wheat straw into animal feed by *Pleurotus ostreatus*. Animal feed Sci. and Techn. 37(2):59-72.

Tiwari, R. K., Jagdish Prasad and A. K. Garg (2007). Variation in chemical composition of different sugarcane crop residues. Tropical Animal Nutrition Conference, National Dairy Research Institute Karnal, India

Van Soest, P.J. and J. Breston (1979). Systems of Analysis for Evaluation Fibrous Feeds In : Standardization of Analytical Methodology for Feed, pp.49, USA.

Williams, A.G. and S.E. Withers.(1985). Formation of polysaccharide depolymerase and glycoside hydrolase enzymes by Bactericides ruminicola subsp. Ruminicola grown in batch and continuous culture. Curr. Micro. 12:79-84. Proceedings of XIV International Congress of Microbiology, Manchester, U.K. Abstract PC 4-4, p. 245.

Zadrazil, F. and H. Brunnert. (1980). The influence of ammonium nitrate supplementation on degradation colonization and in-vitro digestibility of straw colonized by higher fungi. Eur. J. Appl. Microbial. 9: 37-44.

Zadrazil, F.(1977). The conversion of straw into feed by basidiomycetes. Eur. J. Appl. Microbial., 4: 273-281.

تقييم الاختلافات بين بعض بكتريا الكرش بواسطة تقدير معاملات الهضم المعملى للمادة جافة والسيليلوز والهيميسليلوز لمصاصة القصب

هانى محمود جادو ، عتاب رمضان ابراهيم عبد الجليل
قسم الانتاج الحيوانى - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القليوبية - مصر.

يهدف هذا البحث الى تقييم الاختلافات بين خمسة سلالات بكتيرية محللات للسيليلوز وهى

1- *Cellulomonas cellulasea,* 2- *Acetobacter xylinum,* 3- *Ruminococcus albus,*

4- *Thermonospora fusca,* 5- *Bacillus sp.*

حيث تم الحصول على عينات من سائل الكرش بعد الذبح مباشرة للحيوانات فى مجزر البساتين ثم تم عزل هذه السلالات منه مباشرة فى ظروف لاهوائية وذلك من اربعة حيوانات مجترة وهى الابقار والاغنام والجاموس والابل بواسطة تقدير معاملات الهضم المعملية للمادة الجافة والسيليلوز والهيمسيليلوز لمادة مصاصة القصب. ثم تم تحضين مصاصة القصب مع كل سلالة على حدا لمدد ٢-٤-٦-٨-٢٤-٤٨ ساعةحيث استخدم التحضين بعد ٤٨ ساعة للتعبير عن درجة الهضم المعملى (extent) بينما استخدم معامل الانحدار للتعبير عن معدل الهضم المعملى بوحدة / ساعة (rate) .

اوضحت نتائج هذه الدراسة انه بالنسبة لمعاملات الهضم المعملى للمادة الجافة فان قيم معاملات الهضم المعملى للمادة الجافة مع الخمس سلالات البكتيرية المعزولة من الأغنام أكثر تأثيرا عن كل السلالات المعزولة من كلا من الابقار والجاموس والابل . وتعكس النتائج ان بالنسبة لمعاملات الهضم المعملى للسيليلوز ان سلالتين وهما

Cellulomonas cellulasea (65%) and *Ruminococcus albus* (62%) التى تم عزلهم من الابل اعطت اعلى القيم لمعاملات الهضم للسيليلوز لمصاصة القصب و أيضا ان السلالتين المعزولتين من الأبقار وهما *Thermonospora fusca* (56%) and *Acetobacter xylinum* (46%) أظهرت اعلى معاملات هضم معملى للسيليلوزلمصاصة القصب بالمقارنة بالمعزولة من كلا من الابل والأغنام والجاموس.

وتؤكد النتائج ان بالنسبة لمعاملات الهضم المعملى للهيمسيليلوز ان

سلالة *Bacillus sp.* التى تم عزلها من الأغنام اعطت اعلى قيم لمعاملات الهضم لكلا من السيليلوز(53%) والهيمسيليلوز(47%) .

ايضا اوضحت النتائج ان سلالة واحدة معزولة من الابقار وهى *Thermonospora fusca* (52%) أظهرت اعلى معاملات هضم معملى للهيمسيليلوزلمصاصة القصب بينما سلالة واحدة معزولة من الجاموس وهى

Cellulomonas Cellulasea (64%) بالمقارنة بسلالتين من الابل وهما

Acetobacter xylinum (44%) and *Ruminococcus albus* (62%) أظهرت اعلى معاملات هضم معملى للهيمسيليلوزلمصاصة القصب بالمقارنة بالسلالات المعزولة من الأغنام.

مما سبق يمكن استنتاج أنه توجد اختلافات بين السلالات الخمسة المحللة للسيليلوز والمعزولة من اربعة حيوانات مجترة وذلك حيث جميعها تختلف فى معاملات الهضم المعملى للمادة الجافة والسيليلوز والهيمسيليلوز لمصاصة القصب.