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COMPARISON BETWEEN TWO STRAINS OF RUMINAL FIBROLYTIC BACTERIA BY *IN VITRO* DRY MATTER AND ORGANIC MATTER DISAPPEARANCE OF COWPEA HAY.

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SUMMARY

The objective of this research was to study the effect of two strains of fibrolytic bacteria (*Cellulomonas cellulasea* and *Clostridium cellulovorans*) on cowpea hay in anaerobic condition. In the experimental trial bacterial treatments consisted of cowpea hay untreated as control (T1), cowpea hay treated with *Cellulomonas* (T2), cowpea hay treated with *Clostridium* (T3) and cowpea hay treated with a mixture of 1:1 *Cellulomonas* and *Clostridium* (T4). The changes in chemical composition and cell wall constituents were ~~studied in ensiled~~ cowpea hay. Silage was made from cowpea hay incubated for six weeks to investigate effect of replacement CFM by control and bacterial treated cowpea hay (50, 60, 70 and 80%, resulting in rations from R1to R16) on *IVDM* and *IVOM* disappearance from silage. Data showed that treatment of cowpea hay with bacteria significantly ($P<0.05$) decreased CF (43.47 to 32.91%), hemicellulose (28.1 to 20.5%) and cellulose (16.1 to 12.5%) while increased CP (5.92 to12.22%) in all treatments compared with control. The rations containing different percentage of treated cow pea hay with *Cellulomonas* and *Clostridium*, either individually or mixed, increased IVDMD and IVOMD ($P<0.05$) compared to control. Data indicated that IVDMD in R3 and R4 (22.18 and 21.95), which contained 70 and 80% control cow pea hay, were better than R1 (21.18%)with 50% control cow pea hay, but IVOMD in R1, R2, R3 and R4 were not significant in the different experimental rations. While IVDMD in R7 and R8 (29.17 and 29.28%), with content of 70 and 80% treated cow pea hay with *Cellulomonas*, were higher than R5 and R6 (27.01 and 27.96%), which provided 50 and 60% treated cow pea hay. The averages of IVOMD in R8 (38.43%) recorded the highest values compared to R5, R6 and R7 rations. The recorded data showed that averages of IVDMD in R11and R12, that contained 70 and 80% treated cowpea hay (37.70 and 38.51%) were higher than R9 and R10, which provided 50 and 60% treated cowpea hay (35.69 and 36.61%), while IVOMD in R12 was the highest in value (40.99%) compared with R9, R10 and R11 rations. Results revealed that averages of IVDMD in treated cowpea hay with mixed *Cellulomonas* and *Clostridium* (T4) were not significant in R13, R14, R15 and R16 rations. While average of IVOMD in R16 was the highest in value (44.0%) compared with the lowest value in R14 (41.95%) ration. It was concluded that *Clostridium* as a strain of fibrolytic bacteria was more effective than *Cellulomonas* on IVDMD and IVOMD of cowpea hay, especially when rations contained 70 and 80 % treated cowpea hay, except in R15 which recorded the highest value at 70% contents of cowpea hay with *Cellulomonas* and *Clostridium* (T4).

Keywords: *cowpea hay, fibrolytic bacteria, in vitro, dry matter, organic matter.*

INTRODUCTION

Plant cell wall, the major reservoir of fixed carbon in nature, consists of three major polymers: cellulose, hemicellulose, and lignin (Taiz and Zeiger , 1991). In anaerobic environments and decaying plant materials, complex communities of interacting microorganisms carry out the decomposition of lignocellulose. Among the lignocellulolytic bacteria, cellulolytic clostridia play important roles in plant biomass turnover (Leschine, 1995). Cellulose, a long polymer of ß-1,4-glucose, is the major component of plant cell wall (Reiter, 2002). Cellulolytic bacteria and fungi secrete many different types of cellulase enzymes for efficient degradation of this substrate. Many cellulolytic, anaerobic microorganisms secrete multi-enzyme complexes (Bayer,*et al.* 2004, Doi and Tamaru, 2001 and Tardif, *et al.* 2006). The large number and diversity of enzymes secreted by these microorganisms reflect the complex chemical composition of the polysaccharides surrounding the cellulose fibrils in the plant cell wall. Cellulase enzymes are active against numerous substrates, such as crystalline cellulose, the backbone or side chains of xylans, mannans, pectins. The enzymes display various modes of action (endo-, exo-, or processive substrate degradation) (Bayer, *et al.* 2004, Doi and Tamaru ,2001 and Tardif *et al.* 2006). Most of the cellulase enzymes cleave glycosidic bonds by hydrolysis, but a few of them utilize a beta-elimination mechanism (Pagès, *et al.* 2003 and Tardif, *et al.* 2006). Cell wall-degrading enzymes are classified into three distinct groups: glycoside hydrolases, polysaccharide lyases and carbohydrate esterases (Coutinho and Henrissat, 1999).

Sleat *et al.*, (1984) recorded that *Clostridium cellulovorans* and *Cellulomonas cellulasea*, anaerobic bacteria, produces a large number of extracellular polysaccharolytic multi-component complex. *Clostridium cellulovorans* utilizes not only cellulose but also xylan, pectin, and several other carbon sources. Among the available carbon sources, xylan is one of the predominant hemicelluloses in plant cell walls. Abd El-Galil (2000), in a related study, found that biological treatment by *Cellulomonas* of bagasse improved its nutritive value (TDN = 69%) and increased the crude protein from 1.7% to 15.5%. In addition, those bacteria were very active in secreting 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 (2006) indicated that cellulolytic bacteria caused marked increase in crude protein (from 2.59 to15.19%) and decrease in crude fiber (from 46.10 to 27.65%) of corn stalks. All treatments significantly decreased NDF, ADF, cellulose and hemicellulose. In addition, the biological treatments with *Cellulomonas cellulasea* and *Ruminococcus albus* were more effective than other species. However, the treatments increased *IVDMD, IVOMD* and digestibility ($P<0.05$) compared with the untreated corn stalks. The authors concluded that biological treatments with *Cellulomonas cellulasea* and *Ruminococcus albus* in silage improved digestibility and nutritive value of corn stalks. Abd El-Galil (2008) found that using bacterial treatments (*Ruminococcus albus* and *Clostridium cellulovorans)* caused increase in crude protein (from 1.45 to 15.16%) 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, treatments increased *IVDMD, IVOMD* and digestibility ($P<0.05$) compared with the untreated rice straw.

The main goals of the present research are to compare two strains of fibrolytic bacteria isolated from rumen of sheep (*Cellulomonas cellulasea* and *Clostridium cellulovorans*) on chemical composition, cell wall constituents of treated cowpea hay compared with untreated hay. It also compares effect of replacement of DM of CFM in control or treated cowpea hay treated with two strains of bacteria on *In vitro* dry matter and organic matter disappearance.

MATERIALS AND METHODS

Preparation of bacterial cultures:

Two strains of cellulolytic bacteria were isolated from rumen fluid of sheep and were grown as pure cultural. Rumen fluid was collected by stomach tube. The separated strains were *Cellulomonas cellulasea* and *Clostridium cellulovorans*. The isolation of species used the pour-plate technique for pure preparation of cultures according to A.T.C.C. (1992).

Ensiling (small scale silo study):

Cowpea hay was sun dried to 90% DM and chopped to 2-4 cm and mixed with water, molasses, urea (2% w/w), formic acid and acetic acid according to Abd El-Galil (2000). The samples were subjected with one of the following treatments by at1.5 liters (7.7×10^5 viable anaerobes/kg of wet silage)$^{-}$/ton:

T1: Control (untreated) cowpea hay (*Vigna sinesis* hay).

T2: Cowpea treated with *Cellulomonas cellulasea*.

T3: Cowpea treated with *Clostridium cellulovorans*.

T4: Cowpea treated with *Cellulomonas* and *Clostridium* at 1:1 ration.

Treated samples were pressed in 2 liters jars for laboratory use and incubated for 6 weeks.

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, 8, 24 and 48 hrs.).

Proximate analysis:

The proximate analysis of concentrate feed mixture (CFM), control and treated cowpea hay 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. Rate of replacement DM of CFM by cowpea hay (rations) according to the following table:

Item	CFM %			
	50	40	30	20
T1	50 (R1)	60 (R2)	70 (R3)	80 (R4)
T2	50 (R5)	60 (R6)	70 (R7)	80 (R8)
T3	50 (R9)	60 (R10)	70 (R11)	80 (R12)
T4	50 (R13)	60 (R14)	70 (R15)	80 (R16)

Cell wall constituents analysis:

CFM, control and treated cowpea hay 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 difference.

Statistical analysis:

The data of chemical composition, cell wall constituents, *In vitro* dry matter disappearance and *In vitro* organic matter disappearance 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:

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

Where:
Y ij = the observation of the model.
μ = General mean common element to all observation.
Ti = the effect of the treatments (i = 1... 4)
Rk = the effect of rations (k= 1....4)
α ijk = The effect of error.

RESULTS AND DISCUSSION

Chemical composition and cell wall constituents of cowpea hay and concentrate feed mixture (CFM):

Chemical composition and cell wall constituents of concentrate feed mixture (CFM); control (T1) and treated (T2, T3 and T4) cowpea hay are presented in Table (1). Crude protein of cowpea hay increased from 5.92% in control (T1) to 10.53, 12.22 and 11.99 % in T2, T3 and T4, respectively. These effects were due to nitrogen content of added urea (about 2% w/w), microbial protein from bacterial treatments and nitrogen content of growing fibrolytic bacteria in silage of cowpea hay.

Crude fiber decreased from 43.47% in control (T1) to 30.52 % in treated T2, T3 andT4, respectively. The decreasing of crude fiber values in the treatments could be the result of cellulase enzymes secreted by fibrolytic bacteria. Many cellulolytic, anaerobic microorganisms secrete multi-enzyme complexes (Bayer *et al.*, 2004, Doi and Tamaru, 2001 and Tardif *et al.*, 2006). Ash values increased from 12.17% in control (T1) to 15.34, 14.56 and 16.12% in treated T2, T3 and T4, respectively. These effects were due to added media of growing bacteria, molasses and strains of fibrolytic bacteria as bacterial treatments to cowpea hay. In general , it can be concluded, that the two strains of fibrolytic bacteria had great effect (used urea support to building bacterial bodies) on increasing crude protein content (from 5.92 to 12.22%) and increasing degraded crude fiber content (from 43.47 to 30.52 %) of cowpea hay . The highest degradation ($P<0.05$) in NDF content was noted in T3 (36.6%), while the lowest value in NDF content was recorded in T2 (41.7%) compared to T1 (48.5 %). A significant reduction ($P <0.05$) was detected in ADF content from 20.4% in control (Tl) to 17.6, 15.2 and 18.61 % in treated T2, T3 andT4, respectively. On the other side, it was noticed, that hemicellulose content decreased in treated T4 (20.5%) compared to Tl (28.1%). Cellulose content decreased from 16.1 (Tl) to 12.5% in T3 which bacteria were containing capable of degrading the binding between cellulose and other components of degraded cellulose. Lignin content decreased from 4.3 % to 2.7 % with *Clostridium* treatment (T3). In general, results indicated that bacterial treatment by two strains of fibrolytic bacteria with cowpea hay had significant effect on cell wall constituents.

Little research has been done on the extracellular xylanolytic activity in *Clostridium cellulovorans* and *Cellulomonas cellulasea* (Sleat *et al.*, 1984). Those anaerobic bacteria produce a large extracellular polysaccharolytic multi-component complex. *Clostridium cellulovorans* utilizes not only cellulose but also xylan, pectin, and several other carbon sources. Among the available carbon sources, xylan is one of the predominant hemicelluloses in plant cell walls. On the same trend, supportive results confirm the results obtained by Abd El- Galil (2000); (2006) and (2008). Successful utilization of cellulosic materials as renewable carbon sources depends on the development of economically feasible technologies for cellulase production, and for the enzymatic hydrolysis of cellulosic materials to lower the molecular weight products to cellobioses, hexoses, pentoses and glucose (Akihiko Kosugi *et al.*, 2001).

Chemical composition and cell wall constituents of the experimental rations:

Chemical composition and cell wall constituents of CFM replacement by control and treated cowpea hay are presented in Table (2) a and Table (2) b. The data indicated that the crude fiber increased from 28.98% in Rl to 37.67 % in R4. It was also noticed that hemicellulose content increased in R3 and R4. Also, Rl, R2, R3 and R4 recorded, approximately, the same values in cellulose content. The data showed, that the highest value of crude protein was obtained with R5 (13.11 %), while R8, showed the lowest value (11.56%). Crude fiber increased from 25.14% in R5 to 31.52 % in R8. On the other side, it was noticed that hemicellulose content recorded the same values, approximately, in R5, R6, R7 and R8. Treatments R5, R6, R7 and R8 recorded the same values of cellulose content (14%).

The data recorded approximately same values of crude protein for R9, R10, R11 and R12 of 12.92 to 13.96 %. Crude fiber increased from 23.71% in R9 to 29.23 % in R12. Additionally, it was noted *that* hemicellulose content recorded the same values, approximately, in R9, R10, R11 and R12 of around 21%. R9, R10 and R11 recorded the same values of around 13%, while R12 showed the lowest values (12.83%) in cellulose content. Crude protein contents were in R13, R14 and R15 were around 13 %, while R16 showed the lowest value (12.73%). Crude fiber increased from 22.51% in R13 to 27.32 % in R16. Hemicellulose content in R13, R14 and R15 were around 21%, while the lowest value was noted in R16 (20.84%). R14, R 15 and R16 were showed the same values of around 15%, while R13 recorded the lowest value (14.98%) in cellulose content.

In general, it can be concluded that treated cowpea hay increased crude protein content from 11.56 to 13.96% (R5 to R16) compared with control at 7.87 to 10.81% (R1 to R4). Crude fiber content increased from 22.51 to 31.52 % of treated rations compared with control of around 28.98 to 37.67%. Replacement CFM with treated cowpea showed values of hemicellulose around from 20.84 to 23.72 % compared with control around 25.16 to 26.92%. Values of cellulose were around 12.83 to 15.47% in treated rations from R5 to R16 when compared with control.

Table (1). Chemical composition and cell wall constituents of control and treated cowpea hay and concentrate feed mixture (CFM).

Item	CFM	T1 (Control)	T2 *(Cellulomona)*	T3 *(Clostridium)*	T4 *(Cellul+Clost)*	SE
DM	92.10	92.60^{a}	91.40^{b}	91.89^{b}	89.96^{c}	±0.20
OM	91.91	87.83^{a}	84.66^{b}	85.44^{b}	83.88^{c}	±0.18
CP	15.70	5.92^{c}	10.53^{b}	12.22^{a}	11.99^{a}	±0.11
EE	3.22	0.70^{c}	2.70^{a}	2.10^{b}	3.30^{a}	±0.06
CF	14.50	43.47^{a}	35.78^{b}	32.91^{c}	30.52^{d}	±0.10
NFE	58.49	37.74^{a}	35.65^{b}	38.21^{a}	38.07^{a}	±0.23
Ash	8.09	12.17^{c}	15.34^{b}	14.56^{b}	16.12^{a}	±0.04
NDF	42.32	48.50^{a}	41.70^{b}	36.60^{c}	39.11^{b}	±0.15
ADF	20.10	20.40^{a}	17.60^{b}	15.20^{c}	18.61^{b}	±0.18
ADL	5.93	4.30^{a}	3.70^{b}	2.70^{c}	2.81^{c}	±0.12
Hemicell.	22.22	28.10^{a}	24.10^{b}	21.40^{c}	20.50^{c}	±0.37
Cellulose	14.17	16.10^{a}	13.90^{b}	12.50^{c}	15.80^{a}	±0.26

a, b, c & d mean swithin the same row with different superscripts differ significantly (P < 0.05)
Where: CFM: Concentrate feed mixture contained un decorticated cotton seeds 29%, yellow corn 35%, wheat bran 19%, rice bran 7.5%, molasses 4%, lime stone 3.5%, common salt 2%.
T1: Untreated cowpea hay, T2: cowpea treated with Cellulomonas.
T3: cowpea treated with Clostridium, T4: cowpea treated with Cellulomonas and Clostridium.

Table (2a): Chemical composition of concentrate feed mixture (CFM) replacement by control (T1) and treated cowpea hay (T2, T3 and T4)

Item	DM	OM	CP	EE	CF	NFE	Ash
R1	92.35	89.87	10.81^{a}	1.96	28.98^{d}	48.11^{a}	10.13
R2	92.40	89.46	9.83^{a}	1.71	31.88^{c}	46.04^{b}	10.54
R3	92.45	89.05	8.85^{b}	1.45	34.77^{b}	43.96^{c}	10.94
R4	92.50	88.64	7.87^{b}	1.20	37.67^{a}	41.89^{d}	11.35
SE±	0.221	0.191	0.120	0.033	0.110	0.261	0.047
R5	91.75	88.28^{a}	13.11^{a}	2.96	25.14^{d}	47.07^{a}	11.71c
R6	91.68	87.56^{b}	12.59^{a}	2.90	27.27^{c}	44.78^{b}	12.44^{b}
R7	91.61	86.83^{b}	12.08^{a}	2.85	29.39^{b}	42.50^{c}	13.16^{a}
R8	91.54	86.11^{c}	11.56^{b}	2.80	31.52^{a}	40.21^{d}	13.89^{a}
SE±	0.201	0.190	0.130	0.041	0.113	0.262	0.051
R9	91.99	88.67^{a}	13.96^{a}	2.66	23.71^{d}	48.35^{a}	11.33^{c}
R10	91.97	88.03^{a}	13.61^{a}	2.55	25.55c	46.32^{b}	11.97^{b}
R11	91.95	87.38^{b}	13.26^{a}	2.46	27.39^{b}	44.29^{c}	12.62^{a}
R12	91.93	86.73^{b}	12.92^{b}	2.32	29.23^{a}	42.27^{d}	13.27^{a}
SE±	0.192	0.185	0.125	0.042	0.112	0.272	0.052
R13	91.03	87.89^{a}	13.84^{a}	3.26	22.51^{d}	48.28^{a}	12.11^{c}
R14	90.82	87.09^{a}	13.47^{a}	3.27	24.11^{c}	46.24^{b}	12.91^{c}
R15	90.60	86.29^{b}	13.10^{a}	3.28	25.71^{b}	44.19^{c}	13.71^{b}
R16	90.38	85.49^{b}	·12.73^{b}	3.28	27.32^{a}	42.15^{d}	14.51^{a}
SE±	0.188	0.195	0.123	0.054	0.114	0.280	0.060

a, b, c & d means within the same column with different superscripts differ significantly ($P < 0.05$)

Where:

R1: 50% CFM + 50% control cowpea hay (T1), R2: 40% CFM + 60% control cowpea hay (T1)
R3: 30% CFM + 70% control cowpea hay (T1), R4: 20% CFM + 80% control cowpea hay (T1)
R5: 50% CFM + 50% treated cowpea hay (T2), R6: 40% CFM + 60% treated cowpea hay (T2)
R7: 30% CFM + 70% treated cowpea hay (T2), R8: 20% CFM + 80% treated cowpea hay (T2)
R9: 50% CFM + 50% treated cowpea hay (T3), R10: 40% CFM + 60% treated cowpea hay (T3)
R11: 30% CFM + 70% treated cowpea hay (T3), R12: 20% CFM + 80% treated cowpea hay (T3)
R13: 50% CFM + 50% treated cowpea hay (T4), R14: 40% CFM + 60% treated cowpea hay (T4)
R15: 30% CFM + 70% treated cowpea hay (T4), R16: 20% CFM + 80% treated cowpea hay (T4)

Table (2b): Cell wall constituents of concentrate feed mixture (CFM) replacement by control (T1) and treated cowpea hay (T2, T3 and T4)

Item	NDF	ADF	ADL	Hemicellu.	Cellulose
R1	45.41 [c]	20.25	5.11	25.16 [b]	15.13
R2	46.02 [b]	20.28	4.95	25.75 [b]	15.32
R3	46.64 [b]	20.31	4.78	26.33 [a]	15.52
R4	47.26 [a]	20.34	4.62	26.92 [a]	15.71
SE±	0.164	0.166	0.121	0.371	0.270
R5	42.01	18.85	4.81	23.16	14.03
R6	41.94	18.60	4.59	23.34	14.00
R7	41.88	18.35	4.37	23.53	13.98
R8	41.82	18.10	4.14	23.72	13.95
SE±	0.160	0.150	0.120	0.352	0.267
R9	39.46 [a]	17.65 [a]	4.32	21.81	13.34
R10	38.89 [b]	17.16 [a]	3.99	21.73	13.17
R11	38.32 [b]	16.67 [b]	3.67	21.65	13.00
R12	37.74 [c]	16.18 [b]	3.35	21.56	12.83
SE±	0.154	0.155	0.123	0.341	0.265
R13	40.72	19.35	4.37	21.36	14.98
R14	40.39	19.21	4.06	21.19	15.15
R15	40.07	19.06	3.75	21.02	15.31
R16	39.75	18.91	3.43	20.84	15.47
SE±	0.171	0.157	0.121	0.340	0.268

a, b, c & d means within the same column with different superscripts differ significantly ($P < 0.05$)

Where:
R1: 50% CFM + 50% control cowpea hay (T1), R2: 40% CFM + 60% control cowpea hay (T1)
R3: 30% CFM + 70% control cowpea hay (T1), R4: 20% CFM + 80% control cowpea hay (T1)
R5: 50% CFM + 50% treated cowpea hay (T2), R6: 40% CFM + 60% treated cowpea hay (T2)
R7: 30% CFM + 70% treated cowpea hay (T2), R8: 20% CFM + 80% treated cowpea hay (T2)
R9: 50% CFM + 50% treated cowpea hay (T3), R10: 40% CFM + 60% treated cowpea hay (T3)
R11: 30% CFM + 70% treated cowpea hay (T3), R12: 20% CFM + 80% treated cowpea hay (T3)
R13: 50% CFM + 50% treated cowpea hay (T4), R14: 40% CFM + 60% treated cowpea hay (T4)
R15: 30% CFM + 70% treated cowpea hay (T4), R16: 20% CFM + 80% treated cowpea hay (T4)

Effect of replacement CFM by control cowpea hay (T 1) on IVDMD and IVOMD

Data in Table (3) showed that after 48 hrs the highest significant increase ($p<0.05$) was found in R3 and R4 (57.7 and 58.10 %), while the lowest significant increase was noted in R1 (55.6%). IVOMD increased after 48 hrs in R4 (61.0%), while it decreased in R1. Generally, the increase in IVDMD and IVOMD may be due to decrease of CP, CF, NDF, ADF and hemicellulose and cellulose content. From these studies, results of different rations (R1, R2, R3 and R4) indicated that IVDMD in R3 and R4 containing 70 and 80% control cow pea hay (22.18 and 21.95) were better than R1 which provided 50% control cow pea hay (21.18%). averages of IVOMD in R1, R2, R3 and R4 were not significant.

Table (3) : Effect of replacement CFM by control cowpea hay (T 1) on *in vitro* dry and organic matter disappearance

Item	R1	R2	R3	R4	SE
IVDMD (%)					
2hrs	5.30	5.90	5.80	6.10	
4hrs	6.80	7.10	7.20	7.10	
6hrs	8.90	9.00	9.20	9.10	
8hrs	14.70	14.90	15.10	14.50	
24hrs	35.80	37.40	38.10	36.80	
48 hrs	55.60	56.10	57.70	58.10	
average	21.18[b]	21.73[b]	22.18[a]	21.95[a]	±1.10
IVOMD (%)					
2hrs	7.10	7.00	7.20	7.30	
4hrs	9.20	9.40	9.70	9.80	
6hrs	12.80	12.90	13.00	12.70	
8hrs	42.20	42.60	41.10	40.60	
24hrs	52.50	53.00	52.80	51.80	
48 hrs	59.40	60.10	60.20	61.00	
average	30.53	30.83	30.66	30.53	±1.10

a, b, c & d means within the same row with different superscripts differ significantly (P < 0.05)
Where:
R1: 50% CFM + 50% control cowpea hay (T1) *R2: 40% CFM + 60% control cowpea hay (T1)*
R3: 30% CFM + 70% control cowpea hay (T1) *R4: 20% CFM + 80% control cowpea hay (T1)*

Effect of replacement CFM by treated cowpea hay* with *Cellulomonas (T 2) on IVDMD and IVOMD

Data in Table (4) showed that after 48 hrs, the highest value found in R8 (71.10 %), while the lowest value was found in R6 (68.6%). It was noticed, that the values of IVOMD after 48 hrs increased in R8 (77.0%) while it decreased in R5 (72.2%). The increase in IVDMD and IVOMD of rations, which provided different percentages of treated cowpea hay with *Cellulomonas cellulasea* may be due to the decreases of CF, NDF, ADF and hemicellulose and cellulose content and increases of CP compared with rations, which content different percentage of control cowpea hay. From these studies, results of different rations (R5, R6, R7 and R8) indicated that the averages of IVDMD in R7 and R8 containing 70 and 80% treated cow pea hay with *Cellulomonas* (29.17 and 29.28%) were better than the averages of IVDMD in R5 and R6 with only 50 and 60% treated cow pea hay (27.01 and 27.96%). the average of IVOMD in R8 was the highest values (38.43%) compared to R5, R6 and R7 rations.

Generally, these data indicated that the values of IVDMD and IVOMD of rations, which contained different percentages of treated cowpea hay with *Cellulomonas* was the highest in digestibility effect when rations content 70 and 80% (R7 and R8) from treated cowpea hay. The present results are in agreement with Abd El-Galil (2000), who showed that enzymatic treatment (cellulase enzymes) of bagasse improved its chemical composition, cell wall constituents, *In vitro* dry matter and organic matter disappearance.

Abd El-Galil (2006) reported that the proteins with molecular weights of *Cellulomonas cellulasea* ranged 57.000 and 40.000 which were isolated from rumen fluid of baladi goats. This strain secretes cellulase enzymes that break down special cellulasic or lignocellulosic band in cell wall plants. Colombatto *et al.*, (2003) stated that fibrolytic enzymes enhanced the fermentation of cellulose and xylan by a combination of pre- and post- incubation effects. Exogenous fibrolytic enzymes might enhance attachment and /or improve access to the wall matrix by ruminal microorganisms and by doing so, accelerate the rate of digestion (Nsereko *et al.,* 2000). Digestibility of CF increases more with the addition of a certain level of enzyme mixture (2 g/ kg feed) than with lower or higher levels (Yang et al., 1999 and Beauchemin et al., 2000). *Clostridium cellulovorans* degrades native substrates efficiently by producing an extracellular enzyme complex (Roger *et al.*, 2005). Gado and Abd El-Galil,(2009) found that in sheep, the highest active strains on *In vitro* dry matter of bagasse were all strains isolate from rumen of sheep while the highest active strains on *In vitro* hemicellulose disappearance of bagasse were *Bacillus and Cellulomonas*.

Effect of replacement CFM by treated cowpea* hay with *Clostridium (T 3) on IVDMD and IVOMD

Table (5) showed that after 48 hrs, the value of IVDMD in R12 was the highest (80.51%) compared the values in R9, R10 and R11 for 75.7, 78.36 and 78.57 %, respectively. It was clear that the values of IVOMD after 48 hrs increased in R12 (82.50%), while it decreased in R9 (77.32%). However, the increase in IVDMD and IVOMD of rations containing different percentage of treated cowpea hay with *Clostridium cellulovorans* perhaps was due to the decreases of CF, NDF, ADF, hemicellulose , cellulose content and CP increased compared with rations containing different percentage of control cowpea hay. The results indicated that IVOMD in R11and R12 which provided 70 and 80% treated cowpea hay (37.70 and 38.51%) were higher than R9 and R10 (35.69 and 36.61%, respectively). But IVOMD in R12 was the highest in value (40.99%) compared to R9, R10and R11 rations.

It was noticed that the values of IVDMD and IVOMD for rations with different percentage of treated cowpea hay with *Clostridium* was the highest in digestibility, especially when rations containing 80% (R12) from treated cowpea hay.

The present results are agreement with the findings of Abd El-Galil (2000; 2006 and 2008). It is concluded that dry matter digestibility is positively correlated to CP content and negatively correlated to CF, ADF and NDF (Sawe *et al.,* 1998). Gado *et al.* (2009) reported that *Cellulomonas* and *Bacillus* isolated from camel had the highest *In vitro* fermentation of cellulose of bagasse (44.17% and 30.5%). Also, *Cellulomonas* and *Bacillus* isolated from sheep origin recorded *In vitro* fermentation of cellulose of bagasse (42.50% and 30.84%).

Effect of replacement CFM by treated cowpea* hay with *Cellulomonas and Clostridium (T 4) on IVDMD and IVOMD

Effect of replacement CFM with treated cowpea hay (T 4) on *In vitro* dry and organic matter disappearance (R13, R14, R15 and R16) are presented in Table (6).

The highest significant increase of IVDMD ($p<0.05$) were found in R15 (80.71 %), while the lowest significant one was found in R13 (76.72%). The value of IVOMD, after

48 hrs, increased in R15 (86.28%) while it decreased in R13 (80.29%). In these studies, results of different rations indicated that IVDMD in treated cowpea hay with *Cellulomonas* and *Clostridium* (T4) were not significant in R13, R14, R15 and R16 rations. While IVOMD in R16 was the highest value (44.0%) compared the lowest value in R14 (41.95%) rations.

However, the increase in IVDMD and IVOMD of rations containing different percentage of treated cowpea hay with *Cellulomonas* and *Clostridium* was probably due to the noted decreases in CF, CP and hemicellulose content compared with rations content different percentage of control cowpea hay.

Generally, these data indicate that the values of IVDMD and IVOMD of rations providing different percentage of treated cowpea hay with *Cellulomonas* and *Clostridium* were the highest in fermentation when rations provided 70 and 80% (R15 and R16) from treated cowpea hay. These results indicate that *Clostridium*, which isolate from sheep, was more effective than *Cellulomonas* on *In vitro* dry matter and organic matter disappearance of cowpea hay (IVDMD and IVOMD), especially when rations contained 70 and 80 % from treated cowpea hay except in R15 where the highest value was obtained when rations contained 70% from treated cowpea hay with *Cellulomonas* and *Clostridium* (T4).

Table (4) : Effect of replacement CFM by treated cowpea hay (T 2)on *In vitro* dry and organic matter disappearance

Item	R5	R6	R7	R8	SE
IVDMD (%)					
2hrs	8.10	9.50	10.20	10.80	
4hrs	9.60	10.70	11.11	11.50	
6hrs	12.80	12.90	13.50	13.91	
8hrs	19.50	20.80	21.10	21.80	
24hrs	42.40	45.30	48.40	46.60	
48 hrs	69.70	68.60	70.70	71.10	
average	27.01 [b]	27.96[b]	29.17[a]	29.28[a]	±1.09
IVOMD (%)					
2hrs	9.60	9.60	10.40	11.30	
4hrs	11.90	12.20	12.70	13.50	
6hrs	16.80	17.90	16.00	18.70	
8hrs	45.60	47.60	45.10	49.96	
24hrs	54.80	55.00	57.80	60.10	
48 hrs	72.20	73.10	74.20	77.00	
average	35.15[c]	35.90[b]	36.03[b]	38.43[a]	±1.09

a, b, c & d means within the same row with different superscripts differ significantly (P < 0.05)

Where:

R5: 50% CFM + 50% treated cowpea hay (T2), R6: 40% CFM + 60% treated cowpea hay (T2)
R7: 30% CFM + 70% treated cowpea hay (T2), R8: 20% CFM + 80% treated cowpea hay (T2)

Table (5) : Effect of replacement CFM by treated cowpea hay (T 3) on *in vitro* dry and organic matter disappearance

Item	R9	R10	R11	R12	SE
IVDMD (%)					
2hrs	9.91	10.25	10.92	11.82	
4hrs	14.26	13.17	15.22	15.97	
6hrs	19.98	20.19	20.65	22.78	
8hrs	40.35	42.48	43.51	44.38	
24hrs	53.98	55.23	57.34	56.96	
48 hrs	75.70	78.36	78.57	80.51	
average	35.69^{d}	36.61^{c}	37.70^{b}	38.73^{a}	±1.11
IVOMD (%)					
2hrs	10.36	9.60	10.40	11.30	
4hrs	13.19	14.42	14.57	15.85	
6hrs	21.28	22.99	23.60	24.87	
8hrs	41.86	44.76	46.91	50.65	
24hrs	55.38	57.70	58.78	60.81	
48 hrs	77.32	79.41	80.22	82.50	
average	36.56^{d}	38.14^{c}	39.08^{b}	40.99^{a}	±1.11

a, b, c & d means within the same row with different superscripts differ significantly (P < 0.05)
Where: R9: 50% CFM + 50% treated cowpea hay (T3), R10: 40% CFM + 60% treated cowpea hay (T3), R11: 30% CFM + 70% treated cowpea hay (T3), R12: 20% CFM + 80% treated cowpea hay (T3)

Table (6) : Effect of replacement CFM by treated cowpea hay (T 4) on *in vitro* dry and organic matter disappearance

Item	R13	R14	R15	R16	SE
IVDMD (%)					
2hrs	11.91	10.57	10.92	10.88	
4hrs	15.26	15.73	14.47	15.17	
6hrs	20.18	19.92	18.56	17.91	
8hrs	44.55	45.81	39.15	46.82	
24hrs	52.45	54.37	58.42	57.63	
48 hrs	76.72	79.61	80.71	79.11	
average	36.84^{b}	37.67^{a}	37.04^{a}	37.92^{a}	±1.12
IVOMD (%)					
2hrs	12.66	11.62	11.47	12.21	
4hrs	16.92	15.24	16.75	17.35	
6hrs	39.86	36.91	38.04	40.47	
8hrs	50.63	49.67	50.17	51.96	
24hrs	58.84	56.09	57.81	60.11	
48 hrs	80.29	82.15	86.28	81.90	
average	43.20^{b}	41.95^{c}	43.42^{b}	44.0^{a}	±1.12

a, b, c & d means within the same row with different superscripts differ significantly (P < 0.05),

Where: R13: 50% CFM + 50% treated cowpea hay (T4), R14: 40% CFM + 60% treated cowpea hay (T4), R15: 30% CFM + 70% treated cowpea hay (T4), R16: 20% CFM + 80% treated cowpea hay (T4)

Although a large number of microorganisms are capable of degrading cellulose, only few of these microorganisms produce significant quantities of cell-free enzymes capable of completely hydrolyzing crystalline cellulose *In vitro*. A majority of cellulolytic clostridia reported present of several xylanases that have been cloned and characterized (Hayashi *et al.*, 1999; Mohand-Oussaid *et al.*, 1999 and Morag *et al.*, 1990). *Clostridium cellulovorans and Cellulomonas cellulasea*, anaerobic bacteria, produces a large extracellular polysaccharolytic multi-component complex. *Clostridium cellulovorans* utilizes not only cellulose but also xylan, pectin, and several other carbon sources. Among the available carbon sources, xylan is one of the predominant hemicelluloses in plant cell walls (Sleat *et al.*, 1984). Xylanase activity of *Clostridium cellulovorans*, anaerobic cellulolytic bacteria, was identified. Most of the activity was secreted into the growth medium when bacteria were grown on xylan or any carbon sources such as cell wall plants. The major xylanase activities in both fractions were associated with proteins with molecular weights of about 57,000 and 47,000 according to zymogram analyses, and the minor xylanases had molecular weights ranging from 45,000 to 28,000 K Da. (Akihiko Kosugi *et al.*, 2001). Crawford *et al.*, (2004) noticed that cellulases produced as endogenous enzymes are required for degradation of cellulose in plants, and that microbes produce the necessary enzymes. Protein purification from gastric fluid of animal via fast performance liquid chromatography (F P L C) indicated the presence of two endoglucanase enzymes. The molecular weights of these components were determined by matrix-assisted laser desorption /ionization-time of flight (MALDI-TOF) to be 47.887 and 50.295 K Da.

CONCLUSION

These results indicate that under this study *Clostridium* as strain of fibrolytic bacteria, isolate from rumen liquid of sheep, was more effective than *Cellulomonas* on *In vitro* dry matter and organic matter disappearance of cowpea hay (IVDMD and IVOMD) especially when rations provided 70 and 80 % from treated cowpea hay, except in R15 which recorded the highest value when this ration contained 70% from treated cowpea hay with 1:1 of *Cellulomonas* and *Clostridium* (T4). It can be concluded that, the bacterial treatments with *Cellulomonas* and *Clostridium* had more effect on chemical composition and cell wall constituents of treated cowpea hay compared with control untreated cowpea hay. The bacterial treatments with *Cellulomonas* and *Clostridium* could be used to successfully improve the quality of cowpea hay with increased protein, IVDMD and IVOMD, and decreased fibrous fraction of the experimental rations.

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مقارنة بين نوعين من بكتريا الكرش بواسطة تقدير معاملات الهضم المعملي للمادة الجافة والمادة العضوية لتبن اللوبيا

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اجرى هذا البحث بهدف دراسة تأثير معاملة تبن اللوبيا تحت الظروف اللاهوائية كسيلاج باستخدام المعاملة بالبكتريا كما يلى T1 : تبن اللوبيا غير المعامل المقارنة T2: تبن اللوبيا المعامل ببكتريا *Cellulomonas* : T3 تبن اللوبيا المعامل ببكتريا *Clostridium* T4: تبن اللوبيا المعامل بمخلوط من بكتريا *Cellulomonas* و *Clostridium* بنسبة 1:1 لدراسة تأثيرها على التركيب الكيميائى ومكونات جدر خلايا تبن اللوبيا. واستغرقت فترة المعاملة بالبكتريا مدة 6 اسابيع . كما تم دراسة تأثير احلال مخلوط العلف المركز بتبن اللوبيا غير المعامل أو المعامل بنسب مختلفة (50 – 60 – 70 – 80 %) وهى العلائق من R1 الى R16 وتأثيرها على معدل الاختفاء المعملى لكل من المادة الجافة والمادة العضوية لتبن اللوبيا .

وقد اوضحت النتائج ان معاملة تبن اللوبيا بالبكتريا ادى الى خفض الالياف الخام معنويا (من 43,47 الى 32,91 %) والهيمسليلوز (من 28,1 الى 20,5 %) والسيليلوز (من16,10 الى12,50 %) بينما ارتفع البروتين الخام (من 5,92 الى 12,22 %) فى كل المعاملات بالمقارنة بالغير معامل.

واظهرت العلائق المحتوية على نسب مختلفة من تبن اللوبيا المعامل ببكتريا *Cellulomonas* و*Clostridium* ارتفاع كل من معامل الاختفاء المعملى للمادة الجافة والمادة العضوية معنويا مقارنة بعلائق الكنترول .

كما اشارت النتائج الى ارتفاع معامل الهضم المعملى للمادة الجافة معنويا فى العلائق R3 و R4 (22,18 و21,95) والتى تحتوى على 70 % و 80 %من المجموعة المقارنة لتبن للوبيا تعد افضل من معامل الهضم المعملى للمادة الجافة الخاص بالعليقة R1 (21,18 %) والتى تحتوى على 50 % من تبن اللوبيا المقارن مع الاخذ فى الاعتبار انه لاتوجد فروق معنوية بالنسبة لمعاملات هضم المادة العضوية فى العلائق R1 ,R2, R3 R4

ذلك فى الوقت الذى تبين ارتفاع معامل الهضم المعملى للمادة الجافة للعليقتين R7 و R8 (29,17 و 29,28 %) والتى تحتوى على 70% و 80% من تبن اللوبيا المعامل ببكتريا *Cellulomonas* مقارنة بقيم معامل الهضم المعملى للمادة لجافة فى العلائق R5و R6 (27,01 و 27,96)والتى تحتوى على 50 % , 60 %من تبن اللوبيا المعامل . بينما معامل الهضم المعملى للمادة العضوية للعليقة R8 (38,43 %) كان الاعلى مقارنة بالعلائق R5 , R6 , R7.

وقد اوضحت النتائج ان معامل الهضم المعملى للمادة الجافة للعلائق R11 و R12والتى تحتوى على 70 و 80 % من تبن اللوبيا المعامل (37,70 و 38,51 %) كان اعلى من معامل الهضم المعملى للمادة الجافة للعلائق R9 , R10 والتى احتوت على 50 , 60 % من تبن اللوبيا المعامل (35,69 و 36,61) بينما معامل الهضم المعملى للمادة العضوية للعليقة R12 حقق اعلى القيم (40,99 %) مقارنة بالعلائق R9 , R10 , R11 . كما اوضحت النتائج ان قيم معامل الهضم المعملى للمادة الجافة لتبن اللوبيا المعامل بمخلوط من نوعين البكتريا(T4) كان غير معنوى فى العلائق R13 , R14 , R15 , R16.بينما كانت قيمة معامل الهضم المعملى للمادة العضوية للعليقة R16 الاعلى (44,00 %) مقارنة بأقل قيمة للعليقة R14(41,95 %) .

ومما سبق يتضح ان بكتريا *Clostridium* كنوع من انواع البكتريا المحللة للالياف الخام هى الاكثر تأثيرا بالمقارنة ببكتريا *Cellulomonas* وذلك على معامل الهضم المعملى للمادة الجافة والمادة العضوية لتبن اللوبيا خاصة عندما تحتوى العلائق على 70 و 80% من تبن اللوبيا المعامل عدا العليقة R15 والتى سجلت اعلى قيمة عندما احتوت على 70% من تبن اللوبيا المعامل ببكتريا بمخلوط بكتريا *Cellulomonas* و*Clostridium* (T4).