Effect of addition of effective microorganisms on chemical and rumen fermentation characteristics of wheat straw treated with four levels of urea

G.H. Elobied[1], M.T. Yousif[2] and I. A. Abdalla [1]

[1]Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan.
[2]The National Institute for Promotion of Horticultural Exports, University of Gezira, Wad Medani, Sudan.

ABSTRACT

An experiment was conducted in the laboratories of chemistry and biology, University of Gezira, to find the effects of effective microorganisms (EM) on chemical and *in vitro* rumen fermentation characteristics of ammoniated wheat straw on April 2011. Wheat straw was treated with four levels of urea (0, 2, 4 and 6%). Two sets of three replicates each were used. To one set EM was added, while the other one which included no EM was used as a control. It was found that addition of EM increased the urea nitrogen fixed in the straw. CP (crude protein) increased while CF (crude fiber) decreased with increasing levels of urea. The corresponding levels with added EM showed more increase in CP and a decrease in CF, though it was not significant ($P>0.05$). However, at higher urea levels, the straw was significantly ($P<0.05$) improved in terms of increased CP and decreased CF. The DM (dry matter), OM (organic matter), CP and CF digestibilities were improved with increasing levels of urea and it was substantially increased with addition of EM. Rumen fluid pH tended to increase with the increasing levels of urea in both the control and the treatment. Rumen NH_3-N increased significantly ($P<0.05$) at 4% and 6% levels in treatments which included EM and at 6% level in ammoniated straw without EM. Also, there was a trend of increasing total protozoal count with inclusion of EM but this was not significant ($P>0.05$). It could be concluded that inclusion of EM to ammoniated wheat straw improved both the chemical and the *in vitro* rumen fermentation of wheat straw and reduced urea–N lost to the atmosphere.

INTRODUCTION

In Sudan, most ruminant animals depend on grazing natural pastures or fed with cut and carry grasses and crop residues. All the country faces seasonal dry periods in which the availability of pasture decreases and also its quality is seriously affected by the reduction in digestible energy and protein content. Fermentable energy and protein deficiencies in crop residues coupled with their low digestibility impaired the ruminal function, intake and ruminant productivity (Sarwar *et al*., 2004).

Chemical upgrading of low quality roughages by ammonia has been frequently investigated (Sundstol *et al.,* 1978). However, urea treated straw has been known to be superior to untreated straw in terms of crude protein. Spraying layers of straw with urea solution of known concentration achieved the process of upgrading protein of poor quality roughage up to 100% increase (Al-Shami and Al-Sultan, 2006). Different methods may be used for urea treatment of the straw. This included treatment of straw with acidified fermentable carbohydrates (Janxin and Jun, 2002), fixing excess ammonia in the straw by spraying with some organic acids like formic and acetic (Borhami *et al.,* 1982) or inorganic acids like sulphuric and hydrochloric (Cloete and Kritzinger, 1984; Yadav and Virk, 1994a) with different degrees of ammonia fixation. Ammonia liberated from urea as a result of ureolytic organisms is not fully fixed in the straw and abut 60–70% goes to the atmosphere.

Urea and effective microorganisms (EM) was also used in accelerating the decomposition of straw as an organic manure and reduced C: N ratio significantly (Herath *et al.,* 2004). EM is an inoculum of beneficial microorganisms which can co-exist in a mixture. This mixture of microbes consists primarily of photosynthetic and lactic acid bacteria, yeast, actinomycetes and fermenting fungi (Kyan *et al.,* 1999). According to Diver (2001), the main species involved are *Lactobacillus plantarum* L. *casei*, *Streptococcus lactis*, *Rhodopseudomonas plantarum*, *Rhodobacter spaeriodes*, *Saccharomyces cerevisiae* and *Candida utilis*, *Streptomyces albus* and *S. griseus* and *Aspergillus oryzae*, *Pencillium* sp and *Mucar hiemalis*.

Information on the use of EM to improve straw ammonaition (as animal feed) by urea or other ammonia sources is lacking. Therefore, the aim of this study was to investigate the effects of effective microorganisms (EM) and urea treated wheat straw on rumen fermentation characteristics.

MATERIALS AND METHODS

Studies were conducted during April 2011 in the laboratories of biology and chemistry in the University of Gezira, Wad Medani, Sudan. Wheat straw was collected from the University Experimental Farm after wheat harvesting season. Straw was chopped (10-15 cm) and divided into eight sub-samples of 1 kg each in triplicates. The first four sub-samples were treated with 0%, 2%, 4% and 6% urea. While the second four sub-samples which included the same levels of urea, included 0.5% EM solution. EM was added to 500 ml of warm water (37-40^{0}C) to dissolve each urea level and then added to each 1 kg samples of wheat straw and kept in a plastic bag (ensiled in a plastic bag). All samples were kept for three weeks.

The EM solution was kindly provided by Professor Masuzumi, Japan. A sample of 50 g of ensiled straw was used for proximate analysis according to AOAC (1990) and the *in viro* studies. About 5 l of calf (1-2 years) rumen fluid (RF) was obtained from slaughter area in vaccine containers. The fluid was immediately strained through four layers of cheese–cloth. Fermentation studies were conducted according to Kutches *et al*. (1969), where, 100 ml beakers fitted with no. one hole rubber stopper containing a gas release valve were used. In each beaker, 10 g of finally chopped straw (2 -3cm) were put, 10 mls of strained RF and 35 ml of buffer (sodium hydrogen orthophosphate, Na_2CO_3, KCL and

$MgCL_2$) were added. The vessels were swept with CO_2 and the fermentation was allowed to proceed for 24 hours at 39^0C. The microbial activity was stopped by addition of 1 ml of H_2SO_4. RF–pH was read by the use of electrode pH–meter (Mini pH ATC Meter). RF ammonia nitrogen (NH_3-N) was obtained by steam distillation according to AOAC (1990), where, 5ml of RF was mixed with 10ml of NaOH (40%) and steam distillated to obtain NH_3-N concentration (mg/dl).

Total protozoal count was carriedout by diluting 1ml of RF with 9 ml of distilled water (10^{-1}) which was further diluted with 9 ml of distilled water until attaining a dilution of 10^{-8} and protozoa were counted in heamocytometer chamber under the microscope. *In vitro* digestibility was obtained by filtering the fermentation contents through 50 ml sintered glass Gooch crucible and dried at 105^0C in an oven for 24 hrs.

Statistical analysis

Data were subjected to the analysis of variance procedure. Means were separated using Duncan's Multiple Range Test at 5% level of significance.

RESULTS AND DISCUSSION

Effects of urea and EM treatments on straw chemical composition

Inclusion of urea without EM did not affect the straw DM content, while addition of EM significantly ($P<0.05$) reduced the DM (Table 1). This might be attributed to the effect of EM on decomposition rate of the straw and loss of some organic matter (Herath *et al.*, 2004). Zmora–Nahum *et al.* (2007) reported that the OM content in compost produced from wheat straw amounted to about 45%. Also, this is well reflected on the increase in ash and decrease in CF content.

Table 1. Effects of urea and EM on the chemical composition of wheat straw.

Urea level (%)	EM	DM	CP	CF	Ash	NFE	Calculated OM
				(%)			
0.0	-	65.0	4.1	42.1	7.2	46.6	60.3
0.0	+	59.0	3.9	39.0	9.1	48.0	53.6
2.0	-	65.0	5.8	39.6	7.4	47.2	60.2
2.0	+	54.0	6.1	38.6	9.0	46.3	49.1
4.0	-	65.0	7.0	35.4	7.6	50.0	60.1
4.0	+	50.0	8.3	32.6	9.4	49.7	45.3
6.0	-	64.5	7.5	37.1	7.4	48.8	59.7
6.0	+	44.0	9.6	33.0	9.5	47.9	39.8
SE±		2.8	1.4	1.8	1.0	1.1	2.8

EM = Effective microorganisms, DM = Dry matter, CP = Crude Protein, CF = Crude fibre, NFE = Nitrogen free extract, OM = Organic matter

Inclusion of urea significantly ($P<0.05$) increased the protein content of the straw from 4.1 to 7.5 (Table 1). This increase was similar to that reported by other authors (Celik *et al.*, 2003; Mehdikhani *et al.*, 2009). The increase of (0.41–0.83 times) in treatments included urea without EM in this study was lower than that of (1.2-1.4 times) reported by Al–Shami (2008). This may be due to the difference in the incubation period, which was only three weeks in this experiment while Al-Shami (2008)

incubated the straw for eight weeks. However, in the treatment which included EM and urea, the increase in protein (1.13 -1.46 times) was comparable to that reported by Al–Shami (2008).

There was a tendency for CP to increase with increasing urea levels. This increase was not significant ($P>0.05$) when each successive level was compared, though it was substantial even at the lowest level of urea. EM without urea did not affect the level of CP in straw. Addition of EM to 2%, 4% and 6% urea increased the straw bound nitrogen by 47%, 50% and 70%, respectively. However, the optimum bound nitrogen that meets the optimum rumen ammonia for improving rumen environment was reached at 5.5% and 6% for treatments without EM and with EM, respectively (Fig. 1). Urea level of 6%, though it increased the CP significantly ($P<0.01$) when compared to both control and 2% level, it was not significantly ($P>0.05$) different from the 4% level. It was obvious that addition of EM improved straw CP significantly at 6% level of urea. This increase in CP may be attributed to the effect of EM on the decomposition rate of the straw and the reduction of the OM which was reflected on CF decrease and ash increase, in addition to the well established effect of urea treatment on straw CP (Table 1). This coincided with the results of Herath *et al.* (2004) who reported a decrease in C:N ratio as a result of OM decrease due to straw decomposition by the action of EM. In both urea and urea plus EM treatments, reduction of the CF content was not significant except for the treatments which included 4% and 6% urea plus EM. The decline of cell wall constituents in urea ammoniated straw over the control has also been reported earlier (Dass *et al.*, 2000).

It is clear from Table 2 that, the calculated percentage of bound urea nitrogen in wheat straw decreased as the level of urea inclusion increased. The retained NH_3–N from 4% urea level with or without EM was 25–37.4%, respectively. This retained NH_3–N is around to that reported by Saadullah *et al.* (1981) who stated that only 30–35 % of NH_3 released from urea was retained in the straw. The percentage of bound NH_3–N is greater in urea levels which included EM than in the corresponding levels without EM. This may suggest that EM improved straw CP by fixation of excess NH_3–N. Evidence in the literature for the use of EM in NH_3–N fixation in straw is lacking. However, earlier reports showed that excess NH_3-N was fixed by organic acids like formic and acetic (Borhami *et al.*, 1982) or inorganic acids like sulphuric or hydrochloric (Cloete and Kritzinger,1984; Yadav and Virk, 1994) with varying degrees of success.

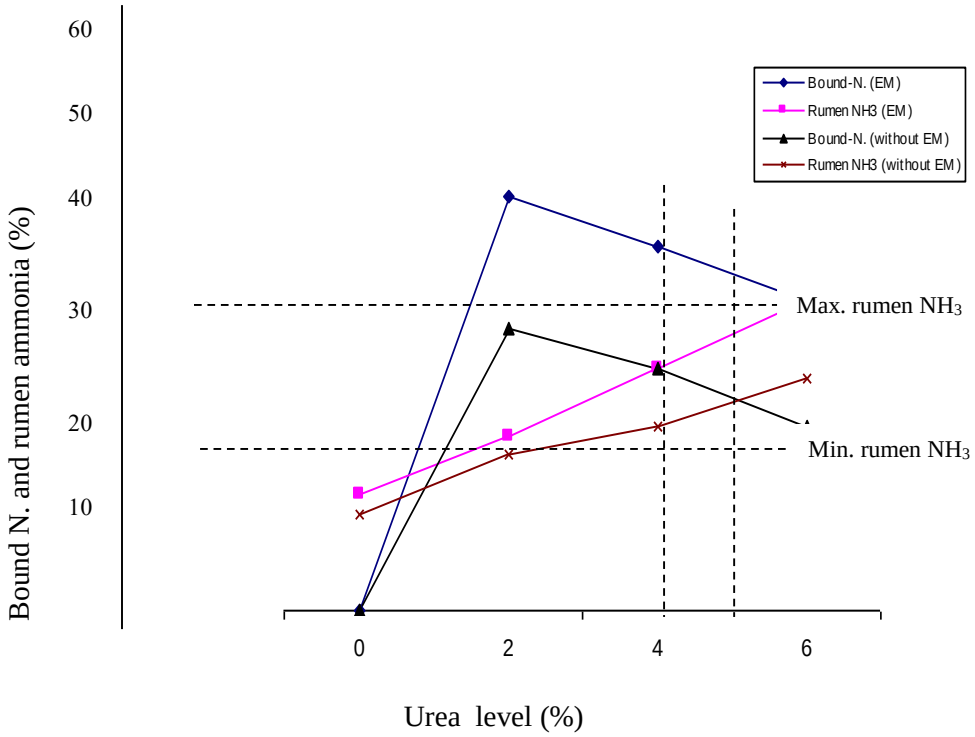

Figure 1. Effects of urea levels on bound N and rumen ammonia.

Table 2. Effects of urea and EM on percentage digestibility of the different components of wheat straw.

Urea level (%)	EM	DM	OM	CP	CF
0	+	42.0	40.0	41.0	30.0
	-	39.5	41.0	39.0	28.0
2	+	48.0	53.0	54.0	37.0
	-	44.5	49.0	48.0	33.0
4	+	54.0	55.0	58.0	42.0
	-	51.5	52.0	51.0	39.0
6	+	53.0	57.0	59.0	44.0
	-	54.0	54.0	53.0	41.0
SE		2.9	2.4	2.7	2.4

EM = Effective microorganisms, DM = Dry matter, CP = Crude Protein,
CF = Crude fibre and OM = Organic matter.

Digestibility of different components of treated straw

Dry matter digestibility increased with increasing levels of urea in both treatments of urea with EM and urea without EM (Table 3). This agreed with the results reported by Milad *et al.* (2010) and Pitchard and Metha (2011). The increase in digestibility is only significant ($P<0.05$) in 4% and 6% urea levels with EM and urea without EM. It is well established that straw DM disappearance increased due

to ammoniation (Caneque *et al.*, 1998; Dass *et al.*, 2000). There was a trend of improving DM digestibility in all levels of urea with addition of EM, but this improvement was not significant (P>0.05) when compared with the corresponding levels of urea without EM.

Table 3. Calculated percent urea-N bound to straw from different urea levels used with or without EM.

Urea level (%)	EM	Urea– N	Straw– N	N bound to straw	N bound (%)	improvement in straw -N
0	-	0.00	0.656	0.000	00.0	0.00
0	+	0.00	0.624	0.000	00.0	0.00
2	-	0.94	0.928	0.272	29.0	41.5
2	+	0.94	1.024	0.400	42.6	64.1
4	-	1.88	1.120	0.464	25.0	70.7
4	+	1.88	1.328	0.704	37.4	112.8
6	-	2.82	1.200	0.544	19.0	82.9
6	+	2.82	1.536	0.912	32.3	146.2

EM = Effective microorganisms.

Values of OMD, CP and CF also followed the same trend of DM digestibility. These results were in line with those reported by Dass *et al.* (1993). Also, Milad *et al.* (2010) reported that ammonia treatment improved *in vitro* OMD values, while Salisbury *et al.* (2004) recorded that N–digestibility increased in ammoniated straw.

Rumen fermentation characteristics

Though there was a trend for rumen pH to increase with increasing levels of urea, but this trend was not significantly (P>0.05) different in both treatments of urea with EM *vs* urea without EM (Table 4). The increase in ruminal pH in both treatments may be ascribed to the effect of NH_3 on rumen pH (Brand *et al.*, 1992). Generally, the rumen fluid pH reported in this study was similar to the range of 6.5–7 reported by Hungate (1966) for maximum microbial growth.

Table 4. Effects of different urea levels treated wheat straw with or without EM on some rumen fermentation characteristics.

Urea level (%)	EM	RF-pH	RF-NH_3-N (mg/dl)	RF-TPC x 10^{-5}
0	+	6.50	16.40	3.25
	-	6.50	12.0	3.25
2	+	6.70	18.0	3.49
	-	7.00	16.0	2.88
4	+	7.10	25.0	3.68
	-	7.00	19.0	2.93
6	+	7.40	32.0	3.06
	-	7.30	24.0	3.02
SE		0.60	2.69	0.54

EM = Effective microorganisms, RF-pH = Rumen fluid pH, RF-NH_3-N = Rumen fluid ammonia nitrogen, RF-TPCx10^{-5}= Rumen fluid total protozoal count.

Rumen fluid NH_3 increased significantly (P<0.05) at 4% and 6% levels in urea plus EM treatment and at 6% in urea without EM treatment. However, the optimum level of rumen ammonia was reached at 4.5% and 6% urea levels in treatments without EM and with EM, respectively (Fig. 1). In all

treatments except the control, NH_3-N was in the range of 15-30 mg/100ml reported by Perdok and Leng (1990) for improving rumen environment. It is clear that NH_3 increased with increasing levels of urea. Similar results were also reported by Pitchard and Metha (2011). However, when EM was added a further increase in NH_3 was noticed.

Total portozoal count (TPC) was neither affected by urea nor EM. However, there was a trend of TPC to increase with inclusion of EM with each urea level. The reason for that is not exactly clear but it may be due to the improvement of the rumen environment. This result might be supported by Al-Shami (2008) who reported that an increase in protozoal concentration and motility may be due to urea feeding. However, Jouney and Ushida (1999) explained that number of protozoa per ml rumen fluid depended on the rate of soluble sugars, starch and rumen pH.

It could be concluded that addition of EM to straw ammoniation with urea improved nitrogen retention, DM, OM, CP and CF digestibilities proportionately to the level of added urea. Also, rumen fermentation characteristics were improved with treatments which included 0.5% EM and urea compared to treatments which included urea without EM.

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أثر إضافة محلول الكائنات الدقيقة الفاعلة على الصفات الكيميائية والتخمر في الكرش لمخلفات محصول القمح المعاملة بأربعة مستويات من اليوريا

قريب الله حسن العبيد[1]، محمد طه يوسف[2] و عصام عدوي عبد الله[1]

[1]كلية العلوم الزراعية ، جامعة الجزيرة ، واد مدني ، السودان.

[2]المعهد القومي لتنمية الصادرات البستانية ، جامعة الجزيرة ، واد مدني ، السودان.

الخلاصة

أجريت هذه التجربة بمعملي الكيمياء والأحياء بجامعة الجزيرة في أبريل 2011م. وذلك بغرض معرفة أثر الكائنات الدقيقة المؤثرة (EM) على الصفات الكيميائية والتخمرية على مخلفات محصول القمح المعاملة بأربعة مستويات من اليوريا (0% ، 2%، 4% و6%). قُسم العلف المعامل باليوريا بالمستويات المختلفة إلى قسمين بثلاث تكرارات لكل قسم (واحد كجم/التكرار). عومل أحد القسمين بـ 0.5% من محلول الكائنات الدقيقة وترك الآخر دون إضافة للكائنات الدقيقة. أظهرت الدراسة أن إضافة الكائنات الدقيقة زاد تثبيت النتروجين في القصب وإزداد البروتين الخام وقلت النسبة المئوية للألياف الخام وذلك بزيادة مستوى اليوريا. المستويات المعاملة بمحلول الكائنات الدقيقة أظهرت زيادة أكثر في هذه العوامل رغم أنها غير معنوية. في المعاملة بالمستويات العليا (4% و6%) من اليوريا مع محلول الكائنات الدقيقة هنالك زيادة معنوية ($P<0.05$) في البروتين الخام ونقصان في الألياف الخام. كما أن هنالك تحسناً في هضمية المادة الجافة ، المادة العضوية ، البروتين الخام والألياف الخام. نشادر الكرش زادت زيادة معنوية ($P<0.05$) في المستويين 4% و6% المعامل بالكائنات الدقيقة أما في القصب المعامل باليوريا فقط كانت الزيادة عند مستوى 6%. هنالك إتجاه رغم عدم معنويته لزيادة أوليات الكرش مع إستخدام محلول الكائنات الدقيقة. أثبتت الدراسة أن إضافة محلول الكائنات الدقيقة للقصب المعامل باليوريا ،حسنت صفاته الكيميائية والتخمرية. وقللت الفاقد من أزوت اليوريا في شكل نشادر.