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EFFECTS OF SLOW-RELEASE UREA ON *IN VITRO* DEGRADATION OF FORAGES

J. A. Sánchez-Meraz[a], S.S. González-Muñoz[b], J.M. Pinos-Rodríguez[c*], Y. López-Hernández[c] and L.A. Miranda[d]

[a] Universidad de la Cañada, Teotitlán de Flores Magón, Oaxaca, 68540, México
[b] Ganadería, Campus Montecillo, Colegio de Postgraduados, Estado de México, 56230, México
[c] Centro de Biociencias, Universidad Autónoma de San Luis Potosí, 78321, México.
[d] Universidad Autónoma Chapingo, Estado de México, 56230, México
[*]Corresponding author Email: jipinos@uaslp.com.mx

ABSTRACT

The objective of this study was to evaluate the *in vitro* effects of slow release urea on gas production and degradation of dry matter and neutral detergent fiber for alfalfa hay, oat straw and corn silage and Stover. The addition of the slow release urea in alfalfa and oat cultures did not improve the degradation of these substrates. However in forages with low content of crude protein, e.g. corn silage and stover, the addition of slow-release urea alone or in combination with urea resulted in a significant increase in degradation and gas production.

Key words: urea, slow-release urea, degradation, gas production, forages.

INTRODUCTION

In ruminants, microbial degradation of fiber and nitrogenous compounds from diet provides 40-100 percent of the amino acids available in the large intestine (Stern *et al*., 1994). Degradation can be improved with the presence of adequate amounts of Nitrogen in the rumen, e.g. 10-16 g/ day (Pisulewski *et al*., 1981). Nitrogen sources for microorganisms are protein N and non-protein N provided by animals diet and recycled salivary urea. In the rumen, the urea is rapidly hydrolyzed releasing ammonia ($N\text{-}NH_3$) which in high concentration may exceed the available energy to synthetize microbial proteins, leading to the intestinal absorption of N-NH3, resulting in an increased urea synthesis in the liver and high levels of urea in blood, affecting the production efficacy and, under certain circumstances, may lead to the ruminant decease (Abdoun *et al*., 2007). Therefore it is important to control de amount of urea supplied in diet, maintaining its levels within 1-3% (Chalupa, 1968). Aiming a slow urea hydrolysis and a sustained nitrogen supply, several compounds have been tested such as biuret, isobutilidene diurea, cereal starch with urea (sterea), and oil coated or Ca+ coupled urea (Currier *et al*., 2004; Huntington *et al*., 2006) with no concluding results since in some cases non-protein nitrogen release is still fast and in others the coupled compounds decrease the hydrolysis or are easily damaged in the rumen resulting in an increased $N\text{-}NH_3$ concentration (Golombeski *et al*., 2006). The use of polymers as urea coatings resulted in a slow release urea (SRU) that can reduce the rumen $N\text{-}NH_3$ generation without affecting the bovine production (Pinos-Rodríguez *et al*., 2010a,b) and may allow an increased amount of urea in diet formulas. The aim of this work was to evaluate the effect of a SRU in the fermentation of alfalfa (*Medicago sativa*) hay, corn (*Zea mays*) silage and Stover and oat (*Avena sativa*) straw by rumen microorganisms *in vitro*.

MATERIALS AND METHODS

Rumen extract was obtained from 650 Kg (live weight) Holstein cows fed with a balanced diet (concentrate and forage 60:40) by a cannula inserted in the rumen. Rumen fluid was processed as standard rumen inoculum (Theodorou *et al*., 1994). Forages were analyzed to determine dry matter, organic matter, crude protein (AOAC, 2003). Samples of forage were boiled (85°C, 2h) using a neutral detergent solution with sodium sulfite (Van Soest *et al*., 1991). The insoluble fraction with the neutral detergent was recovered using a filter (Whatman 541). Insoluble neutral detergent fiber (NDF) was relieved from the filter and then oven dried 24h at 85°C. Insoluble fractions containing 98% of NDF (determined according to Van Soest *et al*., 1991) were used in NDF assays.

To evaluate urea degradation 50 mL of fresh rumen fluid were placed into 100 mL polypropylene tubes, 1 g of Urea or SRU (Optigen, Alltech Inc., Nicholasville, KY, USA) were added and tubes were incubated at 39°C from 0 to 12 hours. After the incubation periods, a sample of 2 ml from each tube was mixed with 0.5 mL of 25% met phosphoric acid, incubated at 4°C for 3 hours and centrifuged at 25000 g for 10 minutes. 20 l of supernatants were mixed with 1 mL of 10 % phenol, 1 mL of NaOH treated sodium hypochlorite and 5 mL of distilled water and incubated at 38°C for 30 minutes. The amount of $N\text{-}NH_3$ was measured by colorimetric assay using a UV-Vis

spectrophotometer (Varian, CARY 1E), according to previously described protocols (McCullough, 1967).

To estimate gas production 90 mL of rumen fluid were placed in each test tube with 0.5 grams of dry matter or neutral detergent fiber and urea and/or SRU (2 and 4 g/100 g dry matter). Tubes were incubated at 37°C and gas pressure readings were performed at 0, 2, 4, 6, 8, 10, 16, 22, 28, 34, 42, 50, 58, 68 and 72 hours using a manometer with scale from 0 to 1 1 kg/cm^2. Values were transformed to gas volume with the linear equation 1

$$V = (P + 0.0186)/(0.0237) \text{ eq. (1)}$$

Where:

V = gas volume

P = gas pressure

With these results the maximum value of gas produced (Vm) and the gas production rate (R) were estimated according to Schofield and Pell (1995) using the NLIN procedure of the statistics SAS package (1999).

To measure the percentage of consumed material, fermentation was stop after 72 hours by freezing the samples and tube residual were recovered by filtration with Number 541 Whatman paper, and dried at 60°C for 24 hours. Cellulolytic bacteria were counted (with a confidence interval of 95%) in cultures of 72 hours with the most probable number method (equation 3, Harrigan and McCance, 1979) and the culture media described by Cobos *et al*. in 2002.

Statistics and analysis of results were done using MIXED and Tukey tests from statistics package SAS, 1999. Result mean values were compared and considered different when $P \leq 0.05$.

RESULTS AND DISCUSSION

In the first 3 hours of rumen fluid culture (with no additional substrate) the concentration of ammonia nitrogen was higher in samples containing urea compared to those with slow release urea (Fig. 1); however from 4 to 12 hours there was no significant difference in the concentration of N-NH_3 in the cultures. Slow release urea may have a slower dilution rate of the urea because of the polymer coat, which could retard the hydrolysis by rumen microorganisms (Abdoun *et al*., 2007).

Gas production (Vm) and substrate degradation of DM and NDF were not influenced by urea and/or SRU addition for alfalfa hay and oat straw maybe because of the high crude protein content of both substrates. For corn silage (DM and NDF) the addition of urea alone resulted in a significant increase in Vm and substrate degradation and with the addition of SRU alone or the combination urea/SRU the increase was significantly higher. However, for corn stover (DM and NDF) the gas production only showed a significant increase with the addition of SRU or urea/SRU and the degradation only increased significantly with the addition of the urea/SRU combination (Table 1). The inconsistence in the positive effects of SRU in the *in vitro* degradation of the forages may be due to the differences between substrates, e.g. the characteristics of each cell wall (Sveinbjörnsson *et al*., 2006).

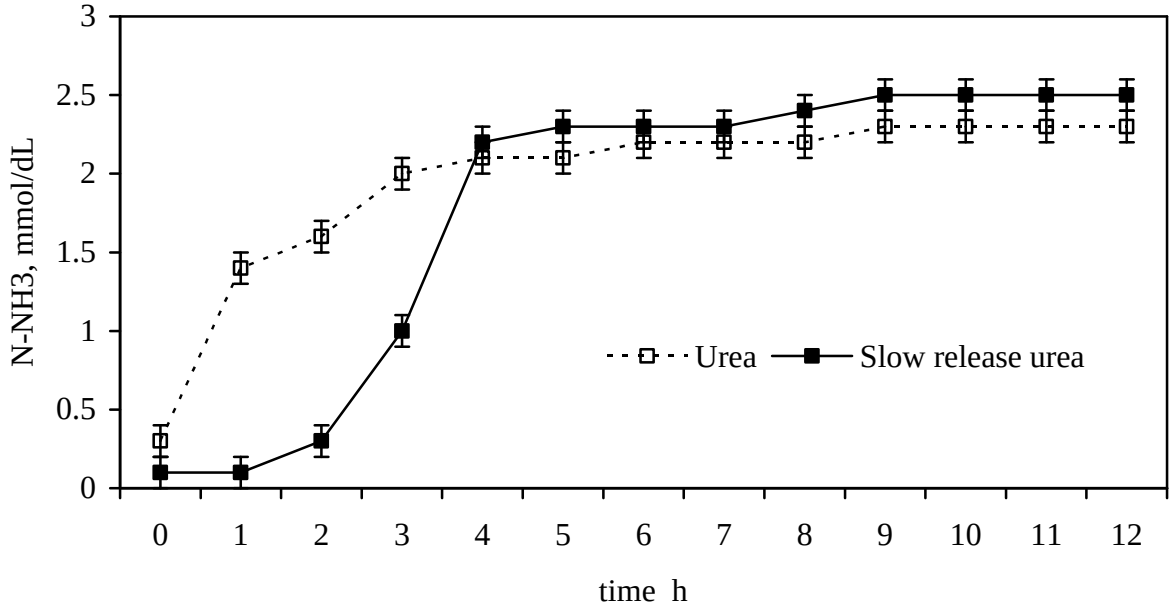

Fig. 1 Ammonia nitrogen (N-NH_3) concentration in cultures of rumen fluid supplemented with urea or slow release urea.

Cellulolytic bacteria concentration was not affected by the addition of Nitrogen sources in alfalfa hay, oat straw and corn stover fermentations (0.5 x10^5/mL; 0.1 to 2.3 x10^5/mL with confidence interval of 95%). However, for corn silage the addition of urea/SRU increased significantly the bacterial concentration from 0.2-5.1 x10^5/mL in control to 0.7 a 15.4 x10^5/mL. It has been shown that the activity of cellulolytic bacteria is favored with increased carbohydrate convenience and continuous availability of ammonia nitrogen (Tedeschi *et al*., 2000) which results in an increased Vm (Nagadi *et al*., 2000). It is likely than the non-structural carbohydrate synchronization together with the slow release of urea increased the gas production observed in corn silage fermentation. The fact that the combination urea/SRU increased the degradation of corn silage and stover suggest a Nitrogen deficiency in these forages, which could disappear with the addition of an effective nitrogen source (Carro *et al*., 1999a,b). Probably the combination of urea /SRU provides both a fast and a continuous source of Nitrogen allowing a synchronization of fast and slow fermentation carbohydrates in the forage.

Table 1 Effect of urea (U) and slow-release urea (SRU) on gas production (Vm, mL/g), gas production rate (R, %/h) and degradation (g/kg) of corn silage and stover, alfalfa hay and oat straw by rumen fluid in 72 h cultures.

Parameter	Raw material	Control	U	SLU	U+SRU	Raw material	Control	U	SLU	U+SRU
Vm	Corn silage Dry matter	251^c	260^b	269^a	272^a	Alfalfa hay Dry matter	237	235	239	241
R		0.036	0.037	0.038	0.037		0.040	0.039	0.039	0.041
Degradation		675^c	689^b	695^a	699^a		691	701	697	695
Vm	Corn silage NDF	211^c	219^b	239^a	243^a	Alfalfa hay NDF	214	217	216	217
R		0.025	0.025	0.027	0.026		0.025	0.025	0.026	0.026
Degradation		395^c	405^b	419^a	420^a		375	370	377	373
Vm	Corn stover Dry matter	276^b	270^b	298^a	302^a	Oat straw Dry matter	251	251	255	257
R		0.031	0.031	0.032	0.032		0.027	0.029	0.028	0.028
Degradation		582^b	580^b	581^b	595^a		589	584	588	587
Vm	Corn stover NDF	250^b	255^b	269^a	274^a	Oat straw NDF	217	213	215	219
R		0.022	0.022	0.022	0.023		0.022	0.022	0.023	0.023
Degradation		403^b	405^b	408^b	422^a		438	433	437	433

Conclusion: The slow-release urea allowed a decrease in the formation of N-NH_3 compared to urea at least in the three first hours of rumen fluid culture. A mixture of urea and slow-release urea increased degradation of forages with low crude protein content.

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