Preservation and Storage of Green Panic (Panicum maximum) as Moist Hay with Urea

N. SILANIKOVE1, O. COHEN2, D. LEVANON3, T. KIPNIS3 and Y. KUGENHEIM2

1MIGAL-Galilee Technological Center, Kiryat Shmona, 10200 (Israel)
2Ministry of Agriculture, Tel Aviv (Israel)
3Agricultural Research Organization, The Volcani Center, Bet Dagan (Israel)

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ABSTRACT


When urea is added to green forage it is hydrolysed and NH3 is released. The possibility of utilizing the fungistatic effect of NH3 to preserve moist Panicum maximum as wet hay with urea was studied at a farm level. Panicum maximum was effectively preserved as wet hay for at least 1 month, which is the typical interval between harvests of perennials. Approximately two-thirds of the applied urea (3.1% urea-N on a dry matter basis) was retained in the material, of which 63% was retained as NH3. Approximately 45% of the retained NH3 and 92% of the retained urea was found in the water-insoluble fraction. Consequently, 62% of the total non-protein-N (NPN) retained was found in the water-insoluble fraction. Fungi and yeasts were mostly eliminated during the storage period, while the number of bacteria was markedly reduced. Dry matter digestibility (DMD) in vitro (70%) in the preserved material was maintained at the level recorded in the green material and this was achieved with minimal loss of organic components.

INTRODUCTION

Feeding cows with freshly-cut green foliage seems to be the most efficient method of exploiting the potential yield and nutritional quality of grasses or legumes from a given field. However, because of organizational problems, this method has been abandoned on most of the large farms in Israel.

Recent works show that urea added to green forage is hydrolysed and ammonia is released (Ghate et al., 1979, 1981). Since NH3 acts as a strong fungistat (Peplinski et al., 1978; Srivastava and Mowat, 1980) and mould fungi are the most common agents which spoil wet forage, treatment with urea resulted in effective preservation. Other potential advantages of the urea treatment are non-protein-N (NPN) enrichment and the improved digestibility of
structural carbohydrates through the alkali effect of the released NH₃ (Dulphy et al., 1984; Ghate et al., 1979, 1981; Huber et al., 1979).

The aims of the present experiment were to: (1) Examine the possibility of scaling-up the method of preserving moist hay with urea to a farm level; (2) Check if the material could be stored in large open stacks and (3) Check if preserved material would be consumed readily by dairy cows and heifers.

EXPERIMENTAL PROCEDURES

Harvesting, preservation and storage

*Panicum maximum* (var. “Galton”) was harvested at the end of May following the first harvest in April. A Hysto Hydroswing 4 m-wide harvester was used to cut the grass without crushing it. Two days later the material was turned over with a rake and left for another day. At the end of this time it reached 60% dry matter (DM). The grass was collected with a forage harvester, chopped into pieces 2–3 cm long, and transferred to a trailer. Urea in the form of pellets (21% N) was dispersed at a calculated rate from a container on to the green material in the trailer in order to provide 35 g urea N kg⁻¹ DM. The treated hay was stored in 2 unsealed stacks.

Samples were taken in triplicate from fresh-cut grass before, and after, urea treatment and from various spots 0.5–1 m deep at a rate of 1 per day for 20 days.

Chemical methods

Moisture, organic matter (OM), ash and N (Kjeldahl) were determined by standard procedures (A.O.A.C., 1980). Cell wall composition (cellulose, hemicellulose and lignin) was determined according to the procedure of Goering and Van Soest (1970), with slight modifications by Silanikove and Levanon (1986) and dry matter digestibility (DMD) was determined in vitro according to Tilley and Terry (1963). Total aerobic bacteria, yeasts and fungi were counted according to the Israel Veterinarian Authority, Animal Feed Standards which are similar to those described by Bothast et al. (1974). Samples were plated in triplicate.

Fractionation of nitrogen of the grass is described in Fig. 1. Material was prepared for analysis by mixing 300 g of the sample with 900 ml of distilled water and homogenizing the material to a particle size of ≤3 mm (Huber et al., 1979). The homogeneous mixture was divided into 4 fractions and treated as follows: (1) Determination of total nitrogen by the Kjeldahl method; (2) Determination of water-soluble urea and water-soluble NH₃ after agitating the sample overnight in dilute acid (1N HCl) and filtering; ammonia was determined by using the distillation and titration steps in the Kjeldahl procedure.
Fig. 1. Scheme for fractionation of non-protein in *Panicum maximum* treated with urea.
From top to bottom: total N = total nitrogen; WS-U = water soluble urea; WS-A = water soluble NH₃; total A = total NH₃; insol-A = insoluble NH₃; total U = total urea; WS-U = water soluble urea; insol-U = insoluble urea.

and urea was determined colorimetrically (Foster and Hocholzer, 1971); (3) Determination of total NH₃ N following dilute acid extraction in the presence of excess MgO (Huber et al., 1979); water-insoluble NH₃ N was calculated by difference between total NH₃ N and water-soluble NH₃ N; (4) Water-insoluble urea was calculated as the difference between total urea (5N HCl extraction) and water-soluble urea.

**Feeding and blood sampling**

Following 20 days of preservation, the treated grass was given to 30 lactating cows and 30 growing heifers. The grass was consumed within 10 days. It contributed 30% and 60% respectively of the total DM intake in the two types of animals. At the end of the feeding period blood samples were randomly taken from 6 animals of each type and from an equal number of control animals. Control animals consumed, instead of preserved grass, an equal proportion (on a DM basis) of wheat silage.

Blood plasma was separated and plasma urea was determined according to Foster and Hocholzer (1971).

**RESULTS**

Green *Panicum maximum* (var. “Galton”) has a high protein content and DMD in vitro (Table I). The structural carbohydrate composition of *P. maximum* is typical of that of grasses in general and includes a large proportion of
hemicellulose (22% of DM) in addition to approximately 35% cellulose. Lignin content (5%) is typical of that found in green material and is considerably lower than that found in typical hay and silage. During the 3 days in which the forage had been left to dry in the field, there was a small reduction in the OM content by residual respiration. This was judged by a 4% increase in ash content ($P < 0.05$) and a 2% and 2.2% decrease in cell solubles and N content, respectively.

Following the preservation treatment with urea, loss of OM content due to residual respiration ceased, as judged by the constancy of ash and cell soluble content. Lignin content in the preserved material was reduced even further (19%, $P < 0.05$) after treatment. Consequently, DMD in vitro was maintained at the high level measured in the grass at harvest (70%, Table I).

Urea degradation began immediately following its application (Fig. 2). Most of the urea degradation occurred within the first 4 days. Parallel to the decrease in urea concentration, NH$_3$ concentration and pH increased. After 8 days the level of urea and NH$_3$ in the stack remained relatively constant (Figs. 2, 3). After 4 days the pH of the material reached its maximum (about 9) and then declined at a low rate (Fig. 4). After 20 days of preservation the pH of the material was still alkaline (about 7.8).

The difference between (total N – protein N) in the original material minus (water-soluble urea–water-soluble NH$_3$) equals the water-insoluble NPN (Fig. 1). The calculated water-insoluble N is larger than the amount of water-insoluble NH$_3$ N, indicating the presence of an additional source of insoluble N. This source may arise from degradation of the plant protein or the presence of water-insoluble urea. Overnight extraction of a homogeneous sample under acidic conditions (5N HCl) resulted in a considerable increase in urea content in comparison with dilute acid extraction.

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**TABLE I**

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>Fresh material</th>
<th>After drying</th>
<th>After storage</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>17.5</td>
<td>66.4</td>
<td>63.9</td>
<td>1.09</td>
</tr>
<tr>
<td>Percentage of DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell solubles</td>
<td>37.4</td>
<td>36.6</td>
<td>36.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Cellulose</td>
<td>34.6</td>
<td>34.8</td>
<td>34.9</td>
<td>0.21</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>22.4</td>
<td>22.6</td>
<td>22.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Lignin</td>
<td>5.40</td>
<td>5.41</td>
<td>4.41$^2$</td>
<td>0.0187</td>
</tr>
<tr>
<td>Ash</td>
<td>12.2</td>
<td>12.6$^2$</td>
<td>12.7$^2$</td>
<td>0.07</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.71</td>
<td>2.65$^2$</td>
<td>4.68$^2$</td>
<td>0.035</td>
</tr>
<tr>
<td>IVDMD</td>
<td>70.5</td>
<td>69.3</td>
<td>70.1</td>
<td>0.57</td>
</tr>
</tbody>
</table>

1Results are given as the mean of samples taken from two stacks.
2Results are significantly different ($P < 0.05$) from the respective value in the fresh material.

Effect of drying *Panicum maximum* in the field for 2 days and storage of treated material for 20 days, on chemical composition (% of DM) and in vitro DMD (IVDMD)
The sum of water-insoluble NH₃ and water-insoluble urea closely matched the amount of water-insoluble NPN. Consequently we were able to quantify all the fractions of NPN retained in the preserved material (Fig. 1, Table II).

After 20 days of preservation, 62.7% of the added urea was recovered as NH₃ and urea. Approximately 63% of the urea was retained in the form of NH₃ and 37% remained unhydrolysed. Approximately 45% of retained NH₃ (28% of the total NPN retained) was found in the water-insoluble fraction, while 92% of the retained urea (34% of the total NPN retained) was water-soluble (Table II). Consequently, 62% of the total NPN retained was found in the water-insoluble fraction.

Fungi and yeasts were mostly eliminated during the storage period with ap-
TABLE II

Retention of non-protein nitrogen (NPN) in Panicum maximum treated with urea (3.1% urea-N on a DM basis) following 20 days of preservation

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>As % of DM</td>
</tr>
<tr>
<td>Nonprotein</td>
<td>2.01 ± 0.3</td>
</tr>
<tr>
<td>Total NH₃</td>
<td>1.26 ± 0.2</td>
</tr>
<tr>
<td>Insoluble NH₃</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>Total urea</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>Insoluble urea</td>
<td>0.69 ± 0.05</td>
</tr>
</tbody>
</table>

1Mean ± SD of 6 samples; 3 samples taken from each stack.

TABLE III

Effect of preservation of Panicum maximum with urea for 20 days on its microbial content (number per g sample ± SD (%))

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fungi</th>
<th>Yeasts</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh material</td>
<td>1.1 × 10¹⁰ ± 10%</td>
<td>none</td>
<td>2.5 × 10¹² ± 12%</td>
</tr>
<tr>
<td>Stack A (3.23% urea)</td>
<td>10 ± 10%</td>
<td>none</td>
<td>5 × 10⁸ ± 15%</td>
</tr>
<tr>
<td>Stack B (3.04% urea)</td>
<td>15 ± 10%</td>
<td>none</td>
<td>5.5 × 10⁸ ± 15%</td>
</tr>
</tbody>
</table>

1Number of colonies developed following plating of the samples in triplicate. The results are related to the DM content of samples to account for the differences between fresh and preserved material.

The NH₃ released by urea hydrolysis effectively preserved the material for at least 30 days, including 20 storage days and 10 days during which the preserved hay was given to the animals.

DISCUSSION

The major obstacle in applying a new process is its scaling-up. In the present investigation the possibility of preserving green grass with urea was demon-
strated in a large-scale field experiment. Preservation was effective for at least 1 month, which is the typical interval between two cuts of a perennial like _P. maximum_. The NH\textsubscript{3} released from urea by hydrolysis and the unhydrolysed urea led to an increase in N content in the water-soluble and water-insoluble fractions.

Approximately two-thirds of the applied urea was retained in the material. Following preservation with either urea or NH\textsubscript{3}, about the same proportion of retained NH\textsubscript{3} (40–50%) was linked to the insoluble fraction (Table II) (see Huber et al., 1979). The presence of insoluble NH\textsubscript{3} in hay was related to adsorption of the NH\textsubscript{3} on cellulose and hemicellulose fractions in the cell wall and to the creation of salts with organic acids in cell solubles (Yahara and Numakawa, 1978).

A considerable amount of the retained urea was not hydrolysed and most of this was retained in the water-insoluble fraction (Table II). Urea is adsorbed on proteins in a stoichiometric matter (see Trochin, 1966 for a review). The enzymatic hydrolysis of urea, however, occurred in the water-soluble fraction. It is assumed, therefore, that adsorption of the retained urea on proteins protected it from enzymatic hydrolysis. In agreement with the above assumption, it was found that when straw (which contains considerably less protein) was treated with urea, 90% or more of the added urea was hydrolysed (Williams et al., 1984a, b).

The greater enrichment of N in the present experiment, in comparison with studies with ammoniation (Huber et al., 1979), is associated with the retention of unhydrolysed urea.

The present results confirmed the results of previous studies (Peplinski et al., 1978; Srivastava and Mowat, 1980; Thorlacius and Robertson, 1984) that NH\textsubscript{3} acts as a lasting fungistat, but suggest that it only temporarily checks bacterial growth. In the work of Srivastava and Mowat (1980) with maize, and of Thorlacius and Robertson (1984) with hay (alfalfa and bromegrass), it was shown that treatment with 2% NH\textsubscript{3} is sufficient to completely prevent mould growth. From the results of the present experiment (Table III), it is concluded that preservation of green forage with 3% urea (30 g urea N kg\textsuperscript{-1} DM) is as effective as 2% NH\textsubscript{3} in preventing mould contamination.

Values for DMD in vitro in the present experiment were maintained at the level recorded in the green material (Table I). A similar capability of maintaining the original digestibility of hay was achieved by treatment with 2% NH\textsubscript{3} and ensiling the treated material (Thorlacius and Robertson, 1984). However, urea treatment is much simpler than ammoniation either with a gas or with a liquid. Ammoniated material should be kept sealed in drums, covered with plastic or ensiled immediately following the treatment. We have shown in the present experiment that the urea-treated material can be stored in large open stacks.

The maintenance of the DMD in vitro at the high level recorded with the
original green material is partially related to the minimal losses by residual respiration during the 3 days that the material was wilted, as judged by the small changes in ash, protein and cell solubles. The main factor affecting the digestibility of both hemicellulose and cellulose (holocellulose) in Panicum appeared to be the extent of lignification (Minson, 1971). According to the equation of Minson (1971) an increase in the extent of lignification from 5% (green and treated grass) to 7.5% (standard hay made from the same source, Silanikove et al., 1986) would reduce holocellulose digestibility from 62% to 50%. As about 50% of Panicum maximum is holocellulose (Table I) such a reduction in holocellulose digestibility would reduce DMD in vitro from 70% to 64%.

In addition, the alkali effect of ammoniation alters the bonding between the structural carbohydrates (cellulose and especially hemicellulose) with lignin, thereby rendering the carbohydrates more digestible (Hartley and Jones, 1978; Van Soest et al., 1983). In addition, Silanikove and Levanon (1987) have noted that alkali treatment probably brings about some degradation of the lignin macromolecule itself in wheat straw and cotton straw. The small decrease in the lignin content which was found in the present experiment in preserved material may represent a response to the alkali effect of NH₃. When P. maximum of lower DMD in vitro (about 60–65%) was treated with urea, digestibility increased (Silanikove et al., 1986). Similarly, it was shown that NH₃ treatment increases intake and digestibility with hays of low feeding value and not with hays of high value (Dulphy et al., 1984).

Additional advantages of the present method of preservation are: (1) rapid removal of the material from the field, allowing immediate continuation of regrowth as well as cultivation practices which enable the maximization of the yield; (2) enrichment of the forage with a cheap source of N which may partly replace more expensive sources of protein; (3) there are no losses of OM by fermentation and effluent which are unavoidable during ensilage, and the losses due to wilting in the field are considerably less than those occurring during drying of hay.

There are numerous reports that ruminants consuming high-quality forage (e.g. sorghum hay and immature grass) treated with anhydrous NH₃ have developed neurological signs, including hyperexcitability, circling and convulsions (Johns et al., 1984; Weiss et al., 1986). Their symptoms developed when relatively large amounts of NH₃ were given (≥3% of DM). No such syndromes were encountered in the present experiment and subsequent application of the method. In the present experiment, NH₃ concentration was stabilized at about 1% of the DM (Fig. 2). There are no reports in the literature on the etiology of ammoniated hay toxicosis at this level of treatment.

Additionally, no problems of palatability of the preserved material and deleterious effects of the NPN on nitrogen status (plasma urea level) were encountered.
Artificially dried, young grass is highly digestible. It is consumed in large amounts and can sustain a substantial milk yield without supplementation (Bines, 1985). However, its high cost relative to other forms of conserved forage precludes its widespread application. In the present work we have shown a way to conserve the quality of high digestibility in young grass cheaply and effectively.

REFERENCES


