A laboratory assessment of various treatment conditions affecting the ammoniation of wheat straw by urea. 1. The effect of temperature, moisture level and treatment period

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The effect of temperature, moisture level and treatment period on the ammoniation of wheat straw by 75 g urea per kg wheat straw was investigated in a 4 x 2 x 6 factorial experiment. Independent variables included temperatures of 4; 14; 24 and 35°C, moisture levels of 250 and 375 g/kg wheat straw and treatment periods of 0; 1; 2; 4; 6 and 8 weeks. Dependent variables investigated were total nitrogen content, urea content, free ammonia nitrogen content and in vitro organic matter digestibility (IVOMD).

Temperature, moisture level and treatment period significantly (P < 0.01) affected ammonia release from urea. The efficiency of ammoniation was accordingly affected. Slow ammonia release at 4°C (at both moisture levels) and at 14°C (only at the lower moisture level) resulted in lesser improvements in IVOMD after a treatment period of eight weeks. Ammoniation was accelerated at higher temperatures, particularly at the higher moisture level. Comparable IVOMD values were obtained after a treatment period of only two weeks at 35°C and approximately six weeks at 24°C. Less ammonia was released from urea at 35°C and the moisture level of 375 g/kg, possibly owing to a decrease in urease activity at this temperature. Significant (P < 0.01) temperature x treatment period and moisture level x treatment period interactions were obtained, indicating that the slow reaction at lower temperatures and at lower moisture levels can partly be compensated for by a longer treatment period.


Die invloed van temperatuur, voegpeil en behandelingsperiode op die ammonifisering van koringstrooi met 75 g ureum per kg strooi is in 'n 4 x 2 x 6-faktoriaal-eksperiment ondersoek. Onafhanklike veranderlikes was temperature van 4; 14; 24 en 35°C, voegpeile van 250 en 375 g/kg strooi, en behandelingsperiodes van 0; 1; 2; 4; 6 en 8 weke. Aangeduide veranderlikes is totale stikstofinvoer, urea-invoer, vry-ammoniak-stikstof-invoer en in vitro-organiese-materiaal-verterbaarheid (IVOMV) behels.

Temperatuur, voegpeil en behandelingsperiode het ammonifisering van koringstrooi beïnvloed. Het ammonifisering van koringstrooi volgens 4°C (bij alle voegpeile) en bij 14°C (slegs bij die laer voegpeil) het geringe stygings in die IVOMV veroorsaak na 'n behandelingstans van agt weke. Ammonifisering by hoër temperatuur, veral by die hoër voegpeil, is versnel. Vergelykbare IVOMV-waardes is verkry na 'n beïnvloeding van die ammonifisering van twee weke by 35°C en ongeeër se weke by 24°C. By 35°C en die voegpeil van 375 g/kg is minder ureum na ammoniak omgesit, waarskynlik as gevolg van 'n verlaagde urease-aktiviteit by die temperatuur. Hoogsbeskikbare voegpeile (P < 0.01) temperatuur x behandelingstans-en voegpeil x behandelingstans-interaksies is waargeneem. 'n Langer behandelingsperiode kan dus tot 'n mate vergoed vir die stadiger ammonifisering wat by lae temperature en lae voegpeile voorkom.


Keywords: Urea ammoniation, temperature, moisture level, treatment period, in vitro organic matter digestibility, nitrogen content

Introduction

The upgrading of low quality roughages by means of ammonia treatment was reviewed by Sundstøl, Coxworth & Mowat (1978). An indirect method of ammoniation of crop residues has recently been reported by Hadjipanayiotou (1982) and Jayasuriya & Perera (1982). This treatment is dependent on the enzymatic release of ammonia from urea added to the roughage in an aqueous medium.

Previous work at this institute proved ammoniation by urea to be effective in improving the in vitro organic matter digestibility of wheat straw (Kritzinger & Franck, 1981). Encouraging results were also obtained in a voluntary intake and in vivo digestibility trial (Cloete, De Villiers & Kritzinger, 1983). This method of ammoniation appears to be relatively safe, uncomplicated and inexpensive when compared to some other chemical treatments. It does, however, require a treatment period of approximately six to eight weeks for optimum results (Kritzinger & Franck, 1981). Results obtained by Oji, Mowat & Winch (1979) and Waagepetersen & Vestergaard Thomsen (1977) indicate that anhydrous ammoniation can effectively be accelerated by treatment at elevated temperatures of up to 90°C. The effect of temperature on the ammoniation of crop residues by urea has so far not been investigated. Literature reviewed by Du Preez (1983), indicated the optimum temperature for urease activity in soil to be approximately 30°C. According to Ørskov, Reid, Holland, Tait & Lee (1983) and Du Preez (1983) urease activity tends to decrease at temperatures lower than 20°C. Soil urease activity, on the other hand, was only marginal at a temperature of 45°C (Wahhab, Khan & Ishaq. 1960).

The effect of moisture level on the ammoniation of low quality roughages was investigated by Sundstøl et al. (1978); Solaiman, Horn & Owens (1979) and Kritzinger & Franck (1981) with moisture levels ranging from 80 to 540 g/kg straw. In general, it can be concluded that ammoniation was consistently faster and more effective at higher moisture levels. Excessive moisture content of treated straw may however present some practical problems under certain circumstances. A laboratory investigation into the effect of temperature on urea ammoniation of wheat straw was thus undertaken. The effect of moisture level, treatment period and the relevant interactions were investigated simultaneously.

Materials and Methods

Ground wheat straw (12 mm screen) was thoroughly mixed with a urea solution to provide a urea level of 75 g urea/kg straw and moisture levels of 250 and 375 g/kg wheat straw.

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Individual samples were sealed airtightly in 2 000 ml plastic containers. Subsequently the urea included in these samples was allowed to react for periods of nil, one, two, four, six and eight weeks at temperatures of 4, 14, 24 and 35°C respectively. Temperatures of 4, 14 and 24°C were obtained by incubation of samples in constant temperature rooms, while an incubator was used for the treatment of samples at 35°C. All samples included in the investigation were treated in duplicate, resulting in a $4 \times 2 \times 6$ factorial design with two replications.

Following treatment the samples were dried at 59°C in a forced-draught oven prior to analyses. Total nitrogen content of the samples was determined by the Kjeldahl method (A.O.A.C., 1970). Samples were also analysed for urea remaining (Technicon Auto Analyser, 1977) and free ammonia nitrogen (Technicon Auto Analyser II, 1977). For these determinations urea and free ammonia nitrogen (NH$_3$-N) were extracted from the respective samples by a 2.0 molar potassium chloride solution containing 5 mg phenyl mercury acetate per litre. In vitro organic matter digestibility (IVOMD) of the samples was determined according to the two-stage technique of Tilley & Terry (1963) as modified by Engels & Van der Merwe (1967). As IVOMD-values were slightly affected by the presence of residual soluble urea in some cases, all these values were corrected for the urea content of the respective samples. Standard procedures for the analyses of a factorial design were applied in the statistical analyses (Snedecor & Cochran, 1967).

**Results and Discussion**

Total nitrogen, urea and free ammonia nitrogen

Degrees of freedom, mean squares and significance for the independent variables, interactions and error in the statistical analyses for total nitrogen content (N content), urea and NH$_3$-N content are presented in Table 1. The effects of temperature, moisture level and treatment period on N content, urea and NH$_3$-N are graphically illustrated in Figures 1, 2 and 3 respectively.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>df</th>
<th>Total N</th>
<th>Urea</th>
<th>NH$_3$-N</th>
<th>IVOMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (T)</td>
<td>3</td>
<td>2,9328*</td>
<td>24,757*</td>
<td>0,35803*</td>
<td>355,01*</td>
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<tr>
<td>Moisture level (M)</td>
<td>1</td>
<td>5,3865*</td>
<td>48,650*</td>
<td>0,20336*</td>
<td>608,084*</td>
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<tr>
<td>Treatment period (P)</td>
<td>5</td>
<td>1,2511*</td>
<td>14,926*</td>
<td>0,19771*</td>
<td>146,314*</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T x M</td>
<td>3</td>
<td>1,3683*</td>
<td>8,055*</td>
<td>0,02067*</td>
<td>58,527*</td>
</tr>
<tr>
<td>T x P</td>
<td>15</td>
<td>0,3389*</td>
<td>2,335*</td>
<td>0,02596*</td>
<td>17,147*</td>
</tr>
<tr>
<td>M x P</td>
<td>5</td>
<td>0,1829*</td>
<td>1,931*</td>
<td>0,00677*</td>
<td>13,354*</td>
</tr>
<tr>
<td>T x M x P</td>
<td>15</td>
<td>0,1277*</td>
<td>0,885*</td>
<td>0,00408*</td>
<td>4,124*</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>0,0053</td>
<td>0,051</td>
<td>0,00052</td>
<td>1,67</td>
</tr>
</tbody>
</table>

* = Significant ($P \leq 0,01$)

Figure 1 The total N content of urea ammoniated wheat straw after various treatment periods at four temperatures and at moisture levels of 250 g/kg wheat straw (a) and 375 g/kg wheat straw (b).

Figure 2 The urea content of urea ammoniated wheat straw after various treatment periods at four temperatures and at moisture levels of 250 g/kg wheat straw (a) and 375 g/kg wheat straw (b).

Figure 3 The free NH$_3$-N content of urea ammoniated wheat straw after various treatment periods at four temperatures and at moisture levels of 250 g/kg wheat straw (a) and 375 g/kg wheat straw (b).

Results obtained indicate that temperature, moisture level and treatment period significantly ($P \leq 0,01$) affected all dependent variables investigated. All interactions were statistically significant ($P \leq 0,01$) too. According to Figures 1 to 3 little urea was converted to ammonia at 4°C at both moisture levels. Higher temperatures and the higher moisture level resulted in accelerated ammonia release, causing corresponding decreases in total N and urea contents (Figures 1 and 2 respectively). These observations resulted in the obtained significant ($P \leq 0,01$) temperature x treatment period and moisture level x treatment period interactions. At 4°C the effect of moisture level was small, resulting in almost no differences between moisture levels. At higher temperatures, con-
sistantly more urea was converted to ammonia at the higher moisture level, causing a significant ($P \leq 0.01$) temperature × moisture level interaction. At 35°C, however, total nitrogen loss was relatively small compared to that at 24°C. One factor contributing to this result was a lower conversion rate of urea to ammonia at 35°C (Figure 2). This trend may possibly be caused by a decline in urease activity at this temperature. Secondly, the NH$_3$-N content of samples ammoniated at 35°C also tended to be higher than that of samples treated at 24°C (Figure 3). It thus appeared that the higher temperature catalysed the binding of extractable ammonia to the straw in some manner, resulting in higher NH$_3$-N levels despite less ammonia released from urea at a temperature of 35°C. At 14°C total N content and urea decreased throughout the entire treatment period. At 24°C and 35°C both these dependent variables tended to reach a minimum level after a treatment period of two to four weeks. Total N content of samples ammoniated at 24°C and 35°C at the higher moisture level, appeared to increase after a treatment period of four weeks. Ammonia bound to the straw as NH$_3$-N (Figure 3) and other nitrogenous compounds possibly contributed to this increase of total N content.

Results in the literature were generally obtained at ambient temperature or temperatures near to 24°C. Results obtained at 24°C and at the higher moisture level (375 g/kg straw) are in general agreement with results obtained by Solaiman et al. (1979) with ammonium hydroxide, and Hadjipanayiotou (1982) and Ibrahim & Pearce (1983) with urea. Solaiman et al. (1979) reported the total N and NH$_3$-N content of wheat straw ammoniated by ammonium hydroxide to be respectively 1.9 and 0.56% after 50 days of treatment. Corresponding values for the present investigation after a treatment period of eight weeks (56 days) were 2.14 and 0.44% respectively. Hadjipanayiotou (1982) investigated the ammoniation of barley straw with 40 g urea/kg straw at a moisture level of 400 g/kg straw. Total N content in his investigation declined from 2.3% after one day to reach a minimum level of 1.15% after 75 days of treatment, while NH$_3$-N increased from 0.1% after one day to 0.47% after 45 days, before levelling off at approximately 0.47%. In the present study, the total N content of wheat straw decreased from 3.77 to a minimum level of 1.56% after two weeks (14 days) of treatment. Subsequently, total N content tended to increase to 2.14% after eight weeks (56 days) of treatment. NH$_3$-N content increased sharply from 0.03% to 0.37% after one week of treatment. A steady increase of NH$_3$-N was observed afterwards, NH$_3$-N reaching a level of 0.44 after eight weeks (56 days) of treatment. The total N content of 1.75% obtained after four weeks (28 days) of treatment in the present investigation agrees fairly well with 1.6% N after 28 days of treatment of barley straw ammoniated by 80 g urea/kg straw and at a moisture level of 1 000 g/kg straw, reported by Ibrahim & Pearce (1983).

**In vitro organic matter digestibility**

Statistical information regarding the effect of temperature, moisture level, treatment period and the relevant interactions on the *in vitro* organic matter digestibility (IVOMD) is presented in Table 1, and graphically illustrated in Figure 4. According to Table 1, IVOMD was significantly ($P \leq 0.01$) affected by temperature, moisture level and treatment period. All the relevant first-order interactions were significant ($P \leq 0.01$) too, while the temperature × moisture level × treatment period interaction was significant at $P \leq 0.05$. Ammoniation for two and one weeks at 35°C and at moisture levels of 250 and 375 g/kg straw respectively, resulted in IVOMD values of approximately 43 and 50%. Corresponding IVOMD values were obtained after a treatment period of six weeks at 24°C for the respective moisture levels. Ammoniation thus appeared to be faster and more effective at 35°C when compared to 24°C, even though less urea was converted to ammonia at the former temperature (Figure 2). Furthermore, wheat straw ammoniated at 35°C also tended to include more NH$_3$-N than that treated at 24°C (Figure 3). These results seem to indicate that the ammoniation reaction was somehow enhanced by the higher temperature. At 4°C the reaction was comparatively slow, resulting in a lesser improvement of IVOMD irrespective of moisture level or treatment period. A corresponding lesser increase was obtained at 14°C and the lower moisture level. At the higher moisture level, on the other hand, the increase of IVOMD appeared to be somewhat more pronounced. No evident explanation exists for the decrease in IVOMD after a treatment period of six weeks at 14°C, and this observation must thus be related to experimental and sampling errors.

The above-mentioned results are in general agreement with those obtained by Waagepetersen & Vestergaard Thomsen (1977), Sundstøl et al. (1978), and Oji et al. (1979). Sundstøl et al. (1978), however, reported a substantial increase in IVOMD of oat straw ammoniated at 4°C. The contradictory results obtained in the present investigation can possibly be explained by the indirect nature of urea ammoniation which is, compared to direct ammoniation, largely dependent on the enzymatic release of ammonia from urea. Owing to the relative inactivity of the urease enzyme at low temperatures (Orskov et al., 1983; Du Preez, 1983) ammonia release at 4°C is slow, resulting in a less efficient ammoniation. The significant ($P \leq 0.01$) interaction between temperature and treatment period, indicating that lower temperatures can partly be compensated for by a longer treatment period, also agrees with results obtained by Sundstøl et al. (1978) and Oji et al. (1979).

Treatment was faster and more effective at the higher moisture level, except at a temperature of 4°C. This result is in agreement with results reported by Sundstøl et al. (1978), Oji et al. (1979), Solaiman et al. (1979), Kiang, Kategile & Sundstøl (1981) and Krieger & Franck (1981). It is also in agreement with results published by Borhami & Sundstøl (1982) on the ammoniation of oat straw. It should, however, be mentioned that Borhami & Sundstøl (1982) investigated comparatively low moisture levels, ranging from 25 to 100 g/kg straw. The interaction between moisture level and treatment period is in agreement with results obtained by Oji et al. (1979). This
result indicates that the slow reaction at low moisture levels can partly be compensated for by a longer treatment period. At 4°C no difference in IVOMD was observed between moisture levels. At higher temperatures ammoniation became increasingly more effective at the higher moisture level, resulting in the observed interaction between temperature and moisture level.

In the present investigation the optimum treatment period at 24°C and at a moisture level of 375 g moisture/kg wheat straw was six to eight weeks. This finding agrees fairly well with results published by Kritzinger & Franck (1981) and Hadjipanayiotou (1982). The former authors reported an optimum treatment period of six to eight weeks for wheat straw ammoniated by urea. Hadjipanayiotou (1982) obtained an improvement in the in vitro dry matter digestibility up to a treatment period of 45 days. Solaiman et al. (1979) and Jayasuriya & Perera (1982), on the other hand, reported little further improvement in the in vitro digestibility of wheat and rice straw, ammoniated by ammonium hydroxide and urea respectively, after respective treatment periods of 10 and 14 days (two weeks).

Conclusions

Temperature markedly affected ammonia release from urea, and accordingly the efficiency of ammoniation. Slow ammonia release at 4°C (at both moisture levels) and at 14°C (only at the lower moisture level) resulted in lesser improvements of IVOMD after a treatment period of eight weeks. At higher temperatures ammoniation was accelerated, particularly at the higher moisture level (375 g/kg straw). Treatment for one to two weeks at 35°C resulted in IVOMD-values comparable to that obtained after a treatment period of six weeks at 24°C, clearly demonstrating the beneficial effect of a rise in temperature on ammoniation as such. On the other hand, slightly less ammonia was released from urea at 35°C and the higher moisture level, indicating that urease activity may be reduced at this temperature. Significant temperature × treatment period and moisture level × treatment period interactions were obtained, indicating that the slow reaction at lower temperatures and at lower moisture levels can partly be compensated for by a longer treatment period.

The obtained results indicate that ammoniation by urea as a method of improving the nutritive value of low quality roughages may not be practical in extremely cold regions, as urease activity tends to be reduced by low temperatures. Extremely high temperatures, on the other hand, may also adversely affect ammonia release from urea, despite having a beneficial effect on ammoniation as such.

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References


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